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I, MARIO PERUSSICH, ASSISTANT DIRECTOR PATENT SERVICES, hereby certify that the annexed are true copies of the Provisional specification and drawing(s) as filed on 14 February 1997 in connection with Application No. PO 5117 for a patent by THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH filed on 14 February 1997.

I further certify that the annexed documents are not, as yet, open to public inspection.

WITNESS my hand this Fourth
day of October 1997

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A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"THERAPEUTIC AND DIAGNOSTIC AGENTS-II"

The invention is described in the following statement:

THERAPEUTIC AND DIAGNOSTIC AGENTS - II

The present invention relates generally to therapeutic and diagnostic agents. More particularly, the present invention provides therapeutic molecules capable of modulating signal transduction such as but not limited to cytokine-mediated signal transduction. The molecules of the present invention are useful, therefore, in modulating cellular responsiveness to cytokines as well as cytokine-mediated immune response mechanisms.

Bibliographic details of the publications referred to in this specification by author are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Cytokines are secreted proteins that regulate the survival, proliferation, differentiation and function of a variety of cells within the body [Nicola, 1994]. The haemopoietic cytokines have in common a four-alpha helical bundle structure and the vast majority interact with a structurally related family of cell surface receptors, the type I and type II cytokine receptors [Bazan, 1990; Sprang, 1993]. In all cases ligand-induced receptor aggregation appears to be a critical event in initiating intracellular signal transduction cascades. Some cytokines, for example growth hormone, erythropoietin (Epo) and granulocyte colony stimulating factor, trigger receptor homodimerisation, while for other cytokines, receptor heterodimerisation or heterotrimerisation is crucial. In the latter cases, several cytokines share common receptor subunits and on this basis can be grouped into three subfamilies with similar patterns of intracellular activation and similar biological effects [Hilton, 1994]. Interleukin-3 (IL-3), IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) use the common β-receptor subunit (βc), and each cytokine stimulates the production and functional activity of

granulocytes and macrophages. IL-2, IL-4, IL-7, IL-9, and IL-15 each use the commonγ-chain (γc), while IL-4 and IL-13 share an alternative γ-chain (γ`c or IL-13 receptor α-chain). Each of these cytokines plays an important role in regulating acquired immunity in the lymphoid system. Finally, IL-6, IL-11, leukaemia inhibitory factor (LIF), oncostatin-M (OSM), ciliary neurotrophic factor (CNTF) and cardiotrophin (CT) share the receptor subunit gp130. Each of these cytokines appears to be highly pleiotropic, having effects both within and outside the haemopoietic system [Nicola, 1994].

In all of the above cases at least one subunit of each receptor complex contains the conserved sequence elements, termed box1 and box2, in their cytoplasmic tails [Murakami, 1991]. Box1 is a proline-rich motif which is located more proximal to the transmembrane domain than the acidic box 2 element. The box-1 region serves as the binding site for a class of cytoplasmic tyrosine kinases termed JAKs (Janus kinases). Ligand-induced receptor dimerisation serves to increase the catalytic activity of the associated JAKs through cross-phosphorylation. Activated JAKs then tyrosine phosphorylate several substrates, including the receptors themselves. Specific phosphotyrosine residues on the receptor then serve as docking sites for SH2-containing proteins, the best characterised of which are the signal transducers and activators of transcription (STATs) and the adaptor protein, shc. The STATs are then phosphorylated on tyrosines, probably by JAKs, dissociate from the receptor and form either homodimers or heterodimers through the interaction of the SH2 domain of one STAT with the phosphotyrosine residue of the other. STAT dimers then translocate to the nucleus where they bind to specific cytokine-responsive promoters and activate transcription [Darnell, 1994; Ihle, 1995; Ihle, 1995]. In a separate pathway, tyrosine phosphorylated shc interacts with another SH2 domain-containing protein, Grb-2, leading ultimately to activation of members of the MAP kinase family and in turn transcription factors such as fos and jun [Sato, 1993; Cutler, 1993]. These pathways are not unique to members of the cytokine receptor family, with cytokines that bind receptor tyrosine kinases also being able to activate STATs and members of the MAP kinase family [David, 1996; Leaman, 1996; Shual, 1993; Sato, 1993; Cutler, 1993].

Four members of the JAK family of cytoplasmic tyrosine kinases have been described, JAK1, JAK2, JAK3 and TYK2, each of which binds to a specific subset of cytokine receptor subunits. Six STATs have been described (STAT1 through STAT6), and these too are activated by distinct cytokine/receptor complexes. For example, STAT1 appears to be functionally specific to the interferon system, STAT4 appears to be specific to IL-12, while STAT6 appears to be specific for IL-4 and IL-13. Thus, despite common activation mechanisms some degree of cytokine specificity may be achieved through the use of specific JAKs and STATs [Thierfelder, 1996; Kaplan, 1996; Takeda, 1996; Shimoda, 1996; Meraz, 1996; Durbin, 1996].

In addition to those described above, there are clearly other mechanisms of activation of these pathways. For example, the JAK/STAT pathway appears to be able to activate MAP kinases independent of the shc-induced pathway [David, 1995] and the STATs themselves can be activated without binding to the receptor, possibly by direct interaction with JAKs [Gupta, 1996]. Conversely, full activation of STATS may require the action of MAP kinase in addition to that of JAKs [David, 1995; Wen, 1995].

While the activation of these signalling pathways is becoming better understood, little is known of the negative feedback loops.

In work leading up to the present invention, the inventors sought to isolate negative regulators of signal transduction. The inventors have now identified a new family of SH2 domain-containing proteins which are capable of acting as regulators of cytokine signalling. The identification of this new family of cytokine regulatory molecules permits the generation of a range of molecules capable of modulating cytokine signal transduction. The present invention provides, therefore, a new family of negative regulators of signal transduction.

The regulatory molecules of the present invention are referred to as suppressors of cytokine signalling or "SOCS". Specific SOCS are defined numerically, for example, SOCS1, SOCS2 and SOCS3.

Accordingly, one aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein suppresses cytokine signal transduction.

More particularly, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein suppresses cytokine signal transduction mediated by at least one of IL-6, LIF, OSM, IFN-γ or thrombopoietin.

Even more particularly, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein suppresses cytokine signal transduction mediated by IL-6 in M1 cells expressing said nucleic acid molecule.

Another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein modulates cellular responsiveness to cytokines.

More particularly, the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein modulates cellular responsiveness to one or more of IL-6, LIF, OSM, IFN-γ or thrombopoietin.

Even more particularly, the present invention relates to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein modulates cellular responsiveness to IL-6 when tested in M1 cells expressing said nucleic acid molecule.

The present invention further contemplates an isolated protein or a derivative thereof comprising a sequence of amino acids having an SH2 domain and which is capable of regulating cytokine signal transduction.

Another aspect provides an isolated protein or a derivative thereof comprising a sequence of amino acids having an SH2 domain and which is capable of regulating cytokine signal transduction for one or more of IL-6, LIF, OSM, IFN-γ or thrombopoietin.

Still another aspect of the present invention is directed to an isolated protein or a derivative thereof comprising a sequence of amino acids having an SH2 domain and which is capable of regulating cytokine signal transduction mediated by IL-6 in M1 cells expressing said protein.

Yet still another aspect of the present invention provides an isolated protein or a derivative thereof comprising a sequence of amino acids having an SH2 domain and which is capable of modulating cellular responsiveness to cytokines.

In a related embodiment, the present invention is directed to an isolated protein or a derivative thereof comprising a sequence of amino acids having an SH2 domain and which is capable of modulating cellular responsiveness to one or more of IL-6, LIF, OSM, IFN-γ or thrombopoietin.

A further embodiment provides an isolated protein or a derivative thereof comprising a sequence of amino acids having an SH2 domain and which is capable of modulating cellular responsiveness to IL-6 when tested in M1 cells expressing said protein or a mimetic of said protein.

In accordance with these aspects of the present invention, the nucleic acid molecules encode a SOCS or a derivative thereof. Reference to a "SOCS" includes reference to family members of SOCS such as SOCS1, SOCS2 and SOCS3. SOCS have an SH2 domain. They

also comprise a C-terminal domain referred to as a C-terminal SOCS domain. Preferably, the SOCS is in recombinant form although the present invention extends to a naturally occurring SOCS in isolated or purified form. The terms "isolated" and "purified" means that a molecule has undergone at least one purification step away from other material.

Preferably, the nucleic acid molecule is in isolated form and is DNA such as cDNA or genomic DNA. The DNA may encode the same amino acid sequence as in the naturally occurring SOCS or the SOCS may contain one or more amino acid substitutions, deletions and/or additions. The nucleotide sequence may correspond to the genomic coding sequence or to the nucleotide sequence in cDNA of the naturally occurring mRNA or may carry one or more nucleotide substitutions, deletions and/or additions thereto.

In one embodiment, the nucleotide sequence of the SOCS, regardless of from which sub-family, comprises the sequence set forth in SEQ ID NO:3 or SEQ ID NO:9 or SEQ ID NO:11 or has at least 15% homology thereto and/or is capable of hybridising the nucleotide sequence set forth in SEQ ID NO:3 under low stringency conditions. The nucleotide sequence set forth in SEQ ID NO:3 encodes mouse SOCS1. SEQ ID NO:9 encodes human SOCS1 and SEQ ID NO:11 encodes rat SOCS1.

In another embodiment, the nucleotide sequence of the SOCS, regardless of from which sub-family, comprises the sequence set forth in SEQ ID NO:5 or has at least 15% homology thereto and/or is capable of hybridising the nucleotide sequence set forth in SEQ ID NO:5 under low stringency conditions. The nucleotide sequence set forth in SEQ ID NO:5 encodes mouse SOCS2.

In a further embodiment, the nucleotide sequence of the SOCS, regardless of from which sub-family, comprises the sequence set forth in SEQ ID NO:7 or has at least 15% homology thereto and/or is capable of hybridising the nucleotide sequence set forth in SEQ ID NO:7 under low stringency conditions. The nucleotide sequence set forth in SEQ ID NO:7 encodes mouse SOCS3.

Within a sub-family, i.e. intra-sub-family, the level of homology is higher, such as at least about 35%.

The present invention further contemplates a nucleotide sequence encoding SOCS1 comprising a sequence set forth in SEQ ID NO:3 or SEQ ID NO:9 or SEQ ID NO:11 or having at least 35% homology thereto and/or is capable of hybridising to the nucleotide sequence of SEQ ID NO:3 or SEQ ID NO:9 and SEQ ID NO:11 under low stringency conditions.

The present invention further contemplates a nucleotide sequence encoding mouse SOCS2 comprising a sequence set forth in SEQ ID NO:5 or having at least 35% homology thereto and/or is capable of hybridising to the nucleotide sequence of SEQ ID NO:5 under low stringency conditions.

The present invention further contemplates a nucleotide sequence encoding mouse SOCS3 comprising a sequence set forth in SEQ ID NO:7 or having at least 35% homology thereto and/or is capable of hybridising to the nucleotide sequence of SEQ ID NO:7 under low stringency conditions.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

In a related embodiment, the nucleotide sequence encodes an amino acid sequence as set forth in SEQ ID NO:4 (referred to herein as SOCS1) or has at least about 15% similarity thereto.

In a further related embodiment, the nucleotide sequence encodes an amino acid sequence as set forth in SEQ ID NO:6 (referred to herein as mouse SOCS2) or has at least about 15% similarity thereto.

In another related embodiment, the nucleotide sequence encodes an amino acid sequence as set forth in SEQ ID NO:8 (referred to herein as mouse SOCS3) or has at least about 15% similarity thereto.

In yet another related embodiment, the nucleotide sequence encodes an amino acid sequence as set forth in SEQ ID NO:10 (referred to herein as human SOCS1) or has at least about 15% similarity thereto.

In yet a further related embodiment, the nucleotide sequence encodes an amino acid sequence as set forth in SEQ ID NO:12 (referred to herein as rat SOCS1) or has at least about 15% similarity thereto.

The above sequence comparisons are preferably to the whole molecule but may also be to part thereof. Preferably, the comparisons are made to a contiguous series of at least about 21 nucleotides or at least about 7 amino acids.

Still another embodiment of the present invention contemplates an isolated polypeptide comprising a sequence of amino acids substantially as set forth in SEQ ID NO:4 or having at least about 15% similarity thereto.

Still another embodiment of the present invention contemplates an isolated polypeptide comprising a sequence of amino acids substantially as set forth in SEQ ID NO:6 or having at least about 15% similarity thereto.

Still another embodiment of the present invention contemplates an isolated polypeptide comprising a sequence of amino acids substantially as set forth in SEQ ID NO:8 or having at least about 15% similarity thereto.

Still another embodiment of the present invention contemplates an isolated polypeptide comprising a sequence of amino acids substantially as set forth in SEQ ID NO:10 or having at least about 15% similarity thereto.

Still another embodiment of the present invention contemplates an isolated polypeptide comprising a sequence of amino acids substantially as set forth in SEQ ID NO:12 or having at least about 15% similarity thereto.

Preferred nucleotide percentage similarities include at least about 20%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or above such as 93%, 95%, 98% or 99%.

Preferred amino acid similarities include at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97% or 98% or above.

The term "similarity" includes exact identity between sequences or, where the sequence differs, different amino acids are related to each other at the structural, functional, biochemical and/or conformational levels.

The nucleic acid molecule may be isolated from any animal such as humans, livestock animals (e.g. horses, cows, sheep, donkeys, pigs), laboratory test animals (e.g. mice, rats, rabbits, hamsters, guinea pigs or primates), companion animals (e.g. dogs, cats) or captive wild animals (e.g. deer, foxes, kangaroos, monkeys and other primates).

The terms "derivatives" or its singular form "derivative" whether in relation to a nucleic acid

molecule or a protein includes parts, mutants, fragments and analogues as well as hybrid or fusion molecules and glycosylation variants. Preferably, the derivatives have functional activity or alternatively act as antagonists or agonists.

One example of an antagonist is an antisense oligonucleotide sequence. Useful oligonucleotides are those which have a nucleotide sequence complementary to at least a portion of the protein-coding or "sense" sequence of the nucleotide sequence. These antisense nucleotides can be used to effect the specific inhibition of gene expression. The antisense approach can cause inhibition of gene expression apparently by forming an antiparallel duplex by complementary base pairing between the antisense construct and the targeted mRNA, presumably resulting in hybridisation arrest of translation.. Ribozymes and co-suppression molecules may also be used. Antibodies may also act as either antagonists or agonists.

The present invention extends to analogues of the SOCS proteins of the present invention and their use in the treatment or prophylaxis of cytokine mediated dysfunction such as autoimmunity, immune suppression or hyperactive immunity or other condition including but not limited to dysfunctions in the haemopoietic, endocrine, hepatic and neural systems. Analogues of the proteins contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 1.

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to

n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

These types of modifications may be important to stabilise the cytokines if administered to an individual or for use as a diagnostic reagent.

TABLE 1

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet

D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp

D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn

L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	Mser	L-α-methylthreonine	Mthr
L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

Other derivatives contemplated by the present invention include a range of glycosylation variants from a completely unglycosylated molecule to a modified glycosylated molecule. Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

Another embodiment of the present invention contemplates a method for modulating expression of a SOCS protein in a mammal, said method comprising contacting a gene encoding a SOCS or a factor/element involved in controlling expression of the SOCS gene with an effective amount of a modulator of SOCS expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of SOCS. An example of a modulator is a cytokine such as IL-6 or other transcription regulators of SOCS expression.

Expression includes transcription or translation or both.

Another aspect of the present invention contemplates a method of modulating activity of SOCS in a human, said method comprising administering to said mammal a modulating effective amount of a molecule for a time and under conditions sufficient to increase or decrease SOCS activity. The molecule may be a proteinaceous molecule or a chemical entity and may also be a derivative of SOCS or a chemical analogue or truncation mutant of SOCS.

A further aspect of the present invention provides a method of inducing synthesis of a SOCS or transcription/translation of a SOCS comprising contacting a cell containing a SOCS gene with an effective amount of a cytokine capable of inducing said SOCS for a time and under conditions sufficient for said SOCS to be produced. For example, SOCS1 may be induced by IL-6.

A further aspect of the present invention contemplates a range of mimetics or small molecules capable of acting as agonists or antagonists of the SOCS. Such molecules may be obtained from natural product screening such as from coral, soil, plants or the ocean or antarctic environments. Alternatively, peptide, polypeptide or protein libraries may be readily screened. For example, M1 cells expressing a SOCS do not undergo proliferation in the presence of IL-6. This system can be used to screen molecules which permit proliferation in the presence of IL-6 and a SOCS. A range of test cells may be prepared to screen for antagonists and agonists for a range of cytokines. Such molecules are preferably small molecules and may be of amino acid origin or of chemical origin.

Accordingly, the present invention contemplates a pharmaceutical composition comprising SOCS or a derivative thereof or a modulator of SOCS expression or SOCS activity and one or more pharmaceutically acceptable carriers and/or diluents. These components are referred to as the "active ingredients".

Although these and other aspects of the present invention apply to any SOCS, one preferred

SOCS is SOCS1. Other preferred SOCS molecules include SOCS2 and SOCS3.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion or may be in the form of a cream or other form suitable for topical application. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in

hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

The present invention also extends to forms suitable for topical application such as creams, lotions and gels.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μg to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating SOCS expression or SOCS activity. The vector may, for example, be a viral

vector.

Still another aspect of the present invention is directed to antibodies to SOCS and its derivatives. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to SOCS or may be specifically raised to SOCS or derivatives thereof. In the case of the latter, SOCS or its derivatives may first need to be associated with a carrier molecule. The antibodies and/or recombinant SOCS or its derivatives of the present invention are particularly useful as therapeutic or diagnostic agents.

For example, SOCS and its derivatives can be used to screen for naturally occurring antibodies to SOCS. These may occur, for example in some autoimmune diseases.. Alternatively, specific antibodies can be used to screen for SOCS. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of SOCS levels may be important for diagnosis of certain cancers or a predisposition to cancers or monitoring cytokine mediated cellular responsiveness or for monitoring certain therapeutic protocols.

Antibodies to SOCS of the present invention may be monoclonal or polyclonal. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for immunotherapy and may also be used as a diagnostic tool for assessing apoptosis or monitoring the program of a therapeutic regimin.

For example, specific antibodies can be used to screen for SOCS proteins. The latter would be important, for example, as a means for screening for levels of SOCS in a cell extract or other biological fluid or purifying SOCS made by recombinant means from culture supernatant fluid. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of SOCS.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the enzyme or protein and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of SOCS, or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art.

Another aspect of the present invention contemplates a method for detecting SOCS in a biological sample from a subject said method comprising contacting said biological sample with an antibody specific for SOCS or its derivatives or homologues for a time and under conditions sufficient for an antibody-SOCS complex to form and then detecting said complex.

The presence of SOCS may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4, 424,279 and 4,018,653. These, of course,

include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain SOCS including cell extract, tissue biopsy or possibly serum, saliva, mucosal secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture.

In the typical forward sandwich assay, a first antibody having specificity for the SOCS or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface Is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for

conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescene and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

The present invention also contemplates genetic assays such as involving PCR analysis to detect SOCS gene or its derivatives. Alternative methods or methods used in conjunction include direct nucleotide sequencing or mutation scanning such as single stranded conformation polymorphisms analysis (SSCP) as specific oligonucleotide hybridisation, as methods such as direct protein truncation tests.

The nucleic acid molecules of the present invention may be DNA or RNA. When the nucleic acid molecule is in DNA form, it may be genomic DNA or cDNA. RNA forms of the nucleic acid molecules of the present invention are generally mRNA.

Although the nucleic acid molecules of the present invention are generally in isolated form, they may be integrated into or ligated to or otherwise fused or associated with other genetic molecules such as vector molecules and in particular expression vector molecules. Vectors and expression vectors are generally capable of replication and, if applicable, expression in one or both of a prokaryotic cell or a eukaryotic cell. Preferably, prokaryotic cells include *E. coli, Bacillus sp* and *Pseudomonas sp.* Preferred eukaryotic cells include yeast, fungal, mammalian and insect cells.

Accordingly, another aspect of the present invention contemplates a genetic construct comprising a vector portion and a mammalian and more particularly a human SOCS gene portion, which SOCS gene portion is capable of encoding a SOCS polypeptide or a functional or immunologically interactive derivative thereof.

Preferably, the SOCS gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of said SOCS gene portion in an appropriate cell.

In addition, the SOCS gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells comprising same.

The present invention also extends to any or all derivatives of SOCS including mutants, part, fragments, portions, homologues and analogues or their encoding genetic sequence including single or multiple nucleotide or amino acid substitutions, additions and/or deletions to the naturally occurring nucleotide or amino acid sequence. The present invention also extends to mimetics and agonists and antagonists of SOCS.

The SOCS and its genetic sequence of the present invention will be useful in the generation of a range of therapeutic and diagnostic reagents and will be especially useful in the detection of a cytokine involved in a particular cellular response or a receptor for that cytokine. For example, cells expressing SOCS gene such as M1 cells expressing the SOCS1 gene, will no longer be responsive to a particular cytokine such as in the case of SOCS1, IL-6. Clearly, the present invention further contemplates cells such as M1 cells expressing the SOCS2 or SOCS3 gene.

Soluble SOCS polypeptides are also contemplated to be particularly useful in the treatment of disease, injury or abnormality involving cytokine mediated cellular responsiveness such as hyperimmunity, immunosuppression, allergies, hypertension and the like.

A further aspect of the present invention contemplates the use of SOCS or its functional derivatives in the manufacture of a medicament for the treatment of conditions involving cytokine mediated cellular responsiveness.

The present invention further contemplates transgenic mammalian cells expressing a SOCS gene. Such cells are useful indicator cell lines for assaying for suppression of cytokine function. One example is M1 cells expressing the SOCS1 gene. Such cell lines may be useful for screening for cytokines or screening molecules such as naturally occurring molecules from plants, coral, microorganisms or bio-organically active soil or water capable of acting as cytokine antagonists or agonists.

The present invention further contemplates hybrids between different SOCS from the same or different animal species. For example, a hybrid may be formed between all or a functional part of mouse SOCS1 and human SOCS1. Alternatively, the hybrid may be between all or part of mouse SOCS1 and mouse SOCS2. All such hybrids are contemplated herein and are particularly useful in developing pleiotropic molecules.

The present invention further contemplates a range of genetic based diagnostic assays screening for individuals with defective SOCS genes. Such mutations may result in cell types not being responsive to a particular cytokine or resulting in over responsiveness leading to a range of conditions. The SOCS genetic sequence can be readily verified using a range of PCR or other techniques to determine whether a mutation is resident in the gene. Appropriate gene therapy or other interventionist therapy may then be adopted.

The present invention is further described by the following non-limiting Figures and Examples.

In the Figures:

Figure 1 is a diagrammatic representation showing generation of an IL-6-unresponsive M1 clone by retroviral infection. The RUFneo retrovirus, showing the position of landmark restriction endonuclease cleavage sites, the 4A2 cDNA insert and the position of PCR primer sequences.

Figure 2 is a photographic representation of Southern and Northern analysis. (Left and Middle Panels) Southern blot analysis of genomic DNA from clone 4A2 and a control infected M1 clone. DNA was digested with BamH I, to reveal the number of retroviruses carried by each clone, and Sac I, to estimate the size of the retroviral cDNA insert. Left panel; probed with neo. Right panel; probed with the Xho I-digested 4A2 PCR product. (Right Panel) . Northern blot analysis of total RNA from clone 4A2 and a control infected M1 clone, probed with the Xho I-digested 4A2 PCR product. The two bands represent unspliced and spliced retroviral transcripts, resulting from splice donor and acceptor sites in the retroviral genome.

Figure 3 is a representation of the nucleotide sequence and structure of the SOCS1 gene. A. The genomic context of SOCS1 in relation to the protamine gene cluster on murine chromosome 16. The accession number of this locus is MMPRMGNS (direct submission; G. Schlueter, 1995) for the mouse and BTPRMTNP2 for the rat (direct submission; G. Schlueter, 1996). B. The nucleotide sequence of the SOCS1 cDNA and deduced amino acid sequence. Conventional one letter abbreviations are used for the amino acid sequence and the asterisk indicates the stop codon. The polyadenylation signal sequence is underlined. The coding region is shown in uppercase and the untranslated region is shown in lower case.

Figure 4 is a graphical representation of cell differentiation in the presence of cytokines. Semi-solid agar cultures of parental M1 cells (M1 and M1.mpl) and M1 cells expressing SOCS1 (4A2 and M1.mpl.SOCS1), were used and the percentage of colonies which differentiated in response to a titration of 1 mg/ml IL-6 ( ), 100 ng/ml LIF (◇), 1 mg/ml

OSM (□), 100 ng/ml IFN-γ (▲), 500 ng/ml TPO (●), or $3x10^{-6}$ M dexamethasone (✻) determined.

Figure 5 is a photographic representation of cytospins of liquid cultures of parental M1 cells (M1 and M1.mpl)) and M1 cells expressing SOCS1 (4A2 and M1.mpl.SOCS1) cultured for 4 days in the presence of 10 ng/ml IL-6 or saline. Unlike parental M1 cells, morphological features consistent with macrophage differentiation are not observed in M1 cells constitutively expressing SOCS1 (4A2 and M1.mpl.SOCS1) when cultured in IL-6.

Figure 6 is a photographic representation showing inhibition of phosphorylation of signalling molecules by SOCS1. Parental M1 cells (M1 and M1.mpl) and M1 cells expressing SOCS1 (4A2 and M1.mpl.SOCS1) were incubated in the absence (-) or presence (+) of 10 ng/ml of IL-6 for 4 minutes at 37°C . Cells were then lysed and extracts were either immunopreciptated using anti-mouse gp130 antibody prior to SDS-PAGE (two upper panels) or were elecectrophoresed directly (two lower panels). Gels were blotted and the filters were then probed with anti-phosphotyrosine (upper panel), anti-gp130 antibody (second top panel), anti-phospho-STAT3 (second bottom panel) or anti-STAT3 (lower panel). Blots were visualised using peroxidase-conjugated secondary antibodies and Enhanced Chemiluminescence (ECL) reagents.

Figure 7 is a representation of protein extracts prepared from (A) M1 cells or M1 cells expressing SOCS1 (4A2) and (B) M1.mpl cells or M1.mpl.SOCS1 cells incubated for 10 min at 37°C in 10 ml serum-free DME containing either saline, 100 ng/ml IL-6 or 100 ng/ml IFN-γ. The binding reactions contained 4-6 μg protein (constant within a given experiment), 5 ng ^{32}P-labelled m67 oligonucleotide encoding the high affinity SIF (c-*sis*-inducible factor) binding site, and 800 ng sonicated salmon sperm DNA. For certain experiments, protein samples were preincubated with an excess of unlabelled m67 oligonucleotide, or antibodies specific for either STAT1 or STAT3.

Figure 8 is a photographic representation of Northern hybridisation. Mice were injected

intravenously with 2 μg and after various periods of time, the livers were removed and polyA+ mRNA was purified. M1 cells were stimulated for various lengths of time with 500 ng/ml of IL-6, after which polyA+ mRNA was isolated. mRNA was fractionated by electrophoresis and immobilized on nylon filters. Northern blots were prehybridised, hybridized with random-primed ^{32}P-labelled SOCS1 or GAPDH DNA fragments, washed and exposed to film overnight.

Figure 9 is a representation of a comparison of the amino acid sequences of SOCS1, SOCS2, SOCS3 and CIS. Alignment of the predicted amino acid sequence of mouse (mm), human (hs) and rat (rr) SOCS1, SOCS2, SOCS3 and CIS. Those residues shaded are conserved in three or four mouse SOCS family members. The SH2 domain is boxed in solid lines, while the SOCS box is bounded by double lines.

Figure 10 is a photographic representation showing the phenotype of IL-6 unresponsive M1 cell clone, 4A2. Colonies of parental M1 cells (left panel) and clone 4A2 (right panel) cultured in semi-solid agar for 7 days in saline or 100 ng/ml IL-6.

Figure 11 is a photographic representation showing expression of mRNA for SOCS family members *in vitro* and *in vivo*.

(A) Northern analysis of mRNA from a range of mouse organs showing constitutive expression of SOCS family members in a limited number of tissues.

(B) Norther analysis of mRNA from liver and M1 cells showing induction of expression of SOCS family members following exposure to IL-6.

(C) Reverse transcriptase PCR analysis of mRNA from bone marrow showing induction of expression of SOCS family members by a range of cytokines.

Figure 12 is a photographic representation showing SOCS1 suppresses the phosphorylation and activation of gp130 and STAT-3.

(A) Western blots of extracts from parental M1 cells (M1 and M1.mpl) and M1 cells expressing SOCS1 (4A2 and M1.mpl.SOCS1) stimulated with (+) or without (-) 100 ng/ml

IL-6. Top: Extracts immunoprecipitated with antu-gp130 (αgp130) and immunoblotted with anti-phosphotyrosine (αPY-STAT3), or for STAT3 (αSTAT3) to demonstrate equal loading of protein. The molecular weights of the bands are shown on the right.

(B) EMSA of M1.mpl and M1.mpl.SOCS1 cells stimulated with (+) and without (-) 100 ng/ml IL-6 or 100 ng/ml IFNγ. The DNA-binding complexes SIF A, B, and C are indicated at the left.

SUMMARY OF SEQUENCE IDENTITY NUMBERS

SEQUENCE	SEQ ID NO.
PCR Primer	1
PCR Primer	2
Mouse SOCS1 (nucleotide)	3
Mouse SOCS1 (amino acid)	4
Mouse SOCS2 (nucleotide)	5
Mouse SOCS2 (amino acid)	6
Mouse SOCS3 (nucleotide)	7
Mouse SOCS3 (amino acid)	8
Human SOCS1 (nucleotide)	9
Human SOCS1 (amino acid)	10
Rat SOCS1 (nucleotide)	11
Rat SOCS1 (amino acid)	12

EXAMPLE 1

CELL CULTURE AND CYTOKINES

The M1 cell line was derived from a spontaneously arising leukaemia in SL mice [Ichikawa, 1969]. Parental M1 cells used in this study have been in passage at the Walter and Eliza Hall Institute for Medical Research, Melbourne, Victoria, Australia, for approximately 10 years. M1 cells were maintained by weekly passage in Dulbecco's modified Eagle's medium (DME) containing 10% (v/v) foetal bovine serum (FCS). Recombinant cytokines are generally available from commercial sources or were prepared by published methods. Recombinant murine LIF was produced in *Escherichia coli* and purified, as previously described [Gearing, 1989]. Purified human oncostatin M was purchased from PeproTech Inc (Rocky Hill, NJ, USA), and purified mouse IFN-γ was obtained from Genzyme Diagnostics (Cambridge, MA, USA). Recombinant murine thrombopoietin was produced as a FLAGTM-tagged fusion protein in CHO cells and then purified.

EXAMPLE 2

AGAR COLONY ASSAYS

In order to assay the differentiation of M1 cells in response to cytokines, 300 cells were cultured in 35 mm Petri dishes containing 1 ml of DME supplemented with 20%(v/v) fetal calf serum (FCS), 0.3%(w/v) agar and 0.1 ml of serial dilutions of IL-6, LIF, OSM, IFN-γ, tpo or dexamethasone (Sigma Chemical Company, St Louis, MI). After 7 days culture at 37°C in a fully humidified atmosphere, containing 10% (v/v) CO_2 in air, colonies of M1 cells were counted and classified as differentiated if they were composed of dispersed cells or had a corona of dispersed cells around a tightly packed centre.

EXAMPLE 3

GENERATION OF RETROVIRAL LIBRARY

A cDNA expression library was constructed from the factor-dependent haemopoietic cell line FDC-P1, essentially as described [Rayner, 1994]. Briefly, cDNA was cloned into the retroviral vector pRUFneo and then transfected into an amphotrophic packaging cell line (PA317). Transiently generated virus was harvested from the cell supernatant at 48 hr

posttransfection, and used to infect Y2 ecotropic packaging cells, to generate a high titre virus-producing cell line.

EXAMPLE 4

RETROVIRAL INFECTION OF M1 CELLS

Pools of 10^6 infected Ψ2 cells were irradiated (3000 rad) and cocultivated with 10^6 M1 cells in DME supplemented with 10%(v/v) FCS and 4 μg/ml Polybrene, for 2 days at 37°C. To select for IL-6-unresponsive clones, retrovirally-infected M1 cells were washed once in DME, and cultured at approximately $2x10^4$ cells/ml in 1 ml agar cultures containing 400 μg/ml geneticin (GibcoBRL, Grand Island, NY) and 100 ng/ml IL-6. The efficiency of infection of M1 cells was 1-2%, as estimated by agar plating the infected cells in the presence of geneticin only.

EXAMPLE 5

PCR

Genomic DNA from retrovirally-infected M1 cells was digested with Sac I and 1 μg of phenol/chloroform extracted DNA was then amplified by polymerase chain reaction (PCR). Primers used for amplification of cDNA inserts from the integrated retrovirus were GAG3 (5' CACGCCGCCCACGTGAAGGC 3' [SEQ ID NO:1]), which corresponds to the vector gag sequence approximately 30 bp 5' of the multiple cloning site, and HSVTK (5' TTCGCCAATGACAAGACGCT 3' [SEQ ID NO:2]), which corresponds to the pMC1neo sequence approximately 200 bp 3' of the multiple cloning site. The PCR entailed an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 2 min, and extension at 72°C for 3 min, followed by a final 10 min extension. PCR products were gel purified and then ligated into the pGEM-T plasmid (Promega, Madison, WI), and sequenced using an ABI PRISM Dye Terminator Cycle Sequencing Kit and a Model 373 Automated DNA Sequencer (Applied Biosystems Inc., Foster City, CA).

EXAMPLE 6

CLONING OF cDNAs

Independent cDNA clones encoding mouse SOCS1 were isolated from a murine thymus cDNA library essentially as described (Hilton *et al,* 1994). The nucleotide and predicted amino acid sequences of mouse SOCS1 cDNA were compared to databases using the BLASTN and TFASTA algorithms (Pearson and Lipman, 1988; Pearson, 1990; Altshcul *et al,* 1990). Oligonucleotides were designed from the ESTs encoding human SOCS1 and mouse SOC-1 and SOCS3 and used to probe commercially available mouse thymus and spleen cDNA libraries. Sequencing was performed using an ABI automated sequencer according to the manufacturer's instructions.

EXAMPLE 7

SOUTHERN AND NORTHERN BLOT ANALYSES AND RT-PCR

^{32}P-labelled probes were generated using a random decanucleotide labelling kit (Bresatec, Adelaide, South Australia) from a 600 bp Pst I fragment encoding neomycin phophotransfease from the plasmid pPGKneo, 1070 bp fragment of the SOCS1 gene obtained by digestion of the 1.4 kbp PCR product with Xho I, SOCS2, SOCS3, CIS and a 1.2 kbp fragment of the chicken glyceraldehyde 3-phosphate dehydrogenase gene [Dugaiczyk, 1983].

Genomic DNA was isolated from cells using a proteinase K-sodium dodecyl sulfate procedure essentially as described. Fifteen micrograms of DNA was digested with either BamH I or Sac I, fractionated on a 0.8%(w/v) agarose gel, transferred to GeneScreenPlus membrane (Du Pont NEN, Boston MA), prehybridised, hybridised with random-primed ^{32}P-labelled DNA fragments and washed essentially as described [Sambrook, 1989].

Total RNA was isolated from cells and tissues using Trizol Reagent, as recommended by the manufacturer (GibcoBRL,Grand Island, NY). When required polyA+ mRNA was purified essentially as described [Alexander, 1995]. Northern blots were prehybridised, hybridized with random-primed 32P-labelled DNA fragments and washed as described [Alexander,

1995].

To assess the induction of SOCS genes by IL-6, mice (C57BL6) were injected intravenously with 5 μg IL-6 followed by harvest of the liver at the indicated timepoints after injection. M1 cells were cultured in the presence of 20 ng/ml IL-6 and harvested at the indicated times. For RT-PCR analysis, bone marrow cells were harvested as described (Metacalf *et al,* 1995) and stimulated for 1 hr at 37°C with 100 ng/ml of a range of cytokines. RT-PCT was performed on total RNA as described (Metcalf *et al,* 1995). PCR products were resolved on an agarose gel and Southern blots were hybridised with probes specific for each SOCS family member. Expression of β-actin was assessed to ensure uniformity of amplification.

EXAMPLE 8

DNA CONSTRUCTS AND TRANSFECTION

A cDNA encoding epitope-tagged SOCS1 was generated by subcloning the entire SOCS1 coding region into the pEF-BOS expression vector [Mizushima, 1990], engineered to encode an inframe FLAG epitope downstream of an initiation methionine (pF-SOCS1). Using electroporation as described previously [Hilton, 1994], M1 cells expressing the thrombopoietin receptor (M1.mpl) were transfected with the 20 μg of Aat II-digested pF-SOCS1 expression plasmid and 2 μg of a Sca I-digested plasmid in which transcription of a cDNA encoding puromycin N-acetyl transferase was driven from the mouse phosphoglycerokinase promoter (pPGKPuropA). After 48 hours in culture, transfected cells were selected with 20 μg/ml puromycin (Sigma Chemical Company, St Louis MO), and screened for expression of SOCS1 by Western blotting, using the M2 anti-FLAG monoclonal antibody according to the manafacturer's instructions (Eastman Kodak, Rochester NY). In other experiments M1 cells were transfected with only the pF-SOCS1 plasmid or a control and selected by their ability to grow in agar in the presence of 100 ng/ml of IL-6.

EXAMPLE 9

IMMUNOPRECIPITATION AND WESTERN BLOTTING

Prior to either immunoprecipitaion or Western blotting, 10^7 M1 cells or their derivatives were washed twice, resuspended in 1ml of DME, and incubated at 37°C for 30 min. The cells were then stimulated for 4 min at 37°C with either saline or 100 ng/ml IL-6, after which sodium vanadate (Sigma Chemical Co., St Louis, MI) was added to a concentration of 1 mM. Cells were placed on ice, washed once with saline containing 1 mM sodium vanadate, and then solubilised for 5 min on ice with 300 μl 1% (v/v) Triton X-100, 150 mM NaCl, 2 mM EDTA, 50 mM Tris-HCl pH 7.4, containing Complete protease inhibitors (Boehringer Mannheim, Mannheim, Germany) and 1 mM sodium vanadate. Lysates were cleared by centrifugation and quantitated using a Coomassie Protein Assay Reagent (Pierce, Rockford IL).

For immunoprecipitations, equal concentrations of protein extracts (1-2 mg) were incubated for 1 hr or overnight at 4°C with either 4 μg of anti-gp130 antibody (M20; Santa Cruz Biotechnology Inc., Santa Cruz, CA) or 4 μg of anti-phosphotyrosine antibody (4G10; Upstate Biotechnology Inc., Lake Placid NY), and 15 μl packed volume of Protein G Sepharose (Pharmacia, Uppsala, Sweden) [Hilton *et al,* 1996]. Immunoprecipitates were washed twice in 1% (v/v) NP40, 150 mM NaCl , 50 mM Tris-HCl pH 8.0, containing Complete protease inhibitors (Boehringer Mannheim, Mannheim, Germany and 1 mM sodium vanadate. The samples were heated for 5 min at 95°C in SDS sample buffer (625 mM Tris-HCl pH 6.8, 0.05% (w/v) SDS, 0.1% (v/v) glycerol, bromophenol blue, 0.125% (v/v) 2-mercaptoethanol), fractionated by SDS-PAGE and immunoblotted as described above.

For Western blotting, 10 μg of protein from a cellular extract or material from an immunoprecipitation reaction was loaded onto 4-15% Ready gels (Bio-Rad Laboratories, Hercules CA), and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to PVDF membrane (Micron Separations Inc., Westborough MA) for 1 hr at 100 V. The membranes were probed with the following

primary antibodies; anti-tyrosine phosphorylated STAT3 (1:1000 dilution; New England Biolabs, Beverly, MA); anti-STAT3 (C-20; 1:100 dilution; Santa Cruz Biotechnology Inc., Santa Cruz CA); anti-gp130 (M20, 1:100 dilution; Santa Cruz Biotechnology Inc., Santa Cruz CA); anti-phosphotyrosine (horseradish peroxidase-conjugated RC20, 1:5000 dilution; Transduction Laboratories, Lexington KY); anti-tyrosine phosphorylated MAP kinase and anti-MAP kinase antibodies (1:1000 dilution; New England Biolabs, Beverly, MA). Blots were visualised using peroxidase-conjugated secondary antibodies and Enhanced Chemiluminescence (ECL) reagents according to the manafacturer's instructions (Pierce, Rockford IL).

EXAMPLE 10

ELECTROPHORETIC MOBILITY SHIFT ASSAYS

Assays were performed as described [Novak, 1995], using the high affinity SIF (c-sis-inducible factor) binding site m67 [Wakao, 1994]. Protein extracts were prepared from M1 cells incubated for 4-10 min at 37°C in 10 ml serum-free DME containing either saline, 100 ng/ml IL-6 or 100 ng/ml IFN-γ. The binding reactions contained 4-6 μg protein (constant within a given experiment), 5 ng ^{32}P-labelled m67 oligonucleotide, and 800 ng sonicated salmon sperm DNA. For certain experiments, protein samples were preincubated with an excess of unlabelled m67 oligonucleotide, or antibodies specific for either STAT1 (Transduction Laboratories, Lexington, KY) or STAT3 (Santa Cruz Biotechnology Inc., Santa Cruz CA), as described [Novak, 1995].

Western blots were performed using anti-tyrosine phosphorylated STAT3 or anti-STAT3 (New England Biolabs, Beverly, MA) or anit-gp130 (Santa Cruz Biotechnology Inc.) as described (Nicola *et al,* 1996). EMSA were performed using the m67 oligonucleotide probe, as described (Novak *et al,* 1995).

EXAMPLE 11

EXPRESSION CLONING OF A NOVEL SUPPRESSOR OF CYTOKINE SIGNAL TRANSDUCTION

In order to identify cDNAs capable of suppressing cytokine signal transduction, an expression cloning approach was adopted. This strategy centred on M1 cells, a monocytic leukaemia cell line that differentiates into mature macrophages and ceases proliferation in response to the cytokines IL-6, LIF, OSM and IFN-γ, and the steroid dexamethasone. Parental M1 cells were infected with the RUFneo retrovirus, into which cDNAs from the factor-dependent haemopoietic cell line FDC-P1 had been cloned. In this retrovirus, transcription of both the neomycin resistance gene and the cloned cDNA was driven off the powerful constitutive promoter present in the retroviral LTR (Figure 1). When cultured in semi-solid agar, parental M1 cells form large tightly packed colonies. Upon stimulation with IL-6, M1 cells undergo rapid differentiation, resulting in the formation in agar of only single macrophages or small dispersed clusters of cells . Retrovirally-infected M1 cells that were unresponsive to IL-6 were selected in semi-solid agar culture by their ability to form large, tightly packed colonies in the presence of IL-6 and geneticin. A single stable IL-6-unresponsive clone, 4A2, was obtained after examining 10^4 infected cells.

A fragment of the neomycin phosphotransferase (neo) gene was used to probe a Southern blot of genomic DNA from clone 4A2 and this revealed that the cell line was infected with a single retrovirus containing a cDNA approximately 1.4 kbp in length (Figure 2). PCR amplification using primers from the retroviral vector which flanked the cDNA cloning site enabled recovery of a 1.4 kbp cDNA insert, which we have named suppressor of cytokine signalling-1, or SOCS1. This PCR product was used to probe a similar Southern blot of 4A2 genomic DNA and hybridised to two fragments, one which corresponded to the endogenous SOCS1 gene and the other, which matched the size of the band seen using the neo probe, corresponded to the SOCS1 cDNA cloned into the integrated retrovirus (Figure 2). The latter was not observed in an M1 cell clone infected with a retrovirus containing an irrelevant cDNA. Similarly, Northern blot analysis revealed that SOCS1 mRNA was abundant in the cell line 4A2, but not in the control infected M1 cell clone (Figure 2).

EXAMPLE 12

SOCS1, SOCS2, SOCS3 AND CIS DEFINE A NEW FAMILY OF SH2-CONTAINING PROTEINS

The SOCS1 PCR product was used as a probe to isolate homologous cDNAs from a mouse thymus cDNA library. The sequence of the cDNAs proved to be identical to the PCR product, suggesting that constitutive or over expression, rather than mutation, of the SOCS1 protein was sufficient for generating an IL-6-unresponsive phenotype. Comparison of the sequence of SOCS1 cDNA with nucleotide sequence databases revealed that it was present on mouse and rat genomic DNA clones containing the protamine gene cluster found on mouse chromosome 16. Closer inspection revealed that the 1.4 kb SOCS1 sequence was not homologous to any of the protamine genes, but rather represented a previously unidentified open reading frame located at the extreme 3′ end of these clones (Figure 3). There were no regions of discontinuity between the sequences of the SOCS1 cDNA and genomic locus, suggesting that SOCS1 is encoded by a single exon. In addition to the genomic clone containing the protamine genes, a series of murine and human expressed sequenced tags (ESTs) also revealed large blocks of nucleotide sequence identity to mouse SOCS1. The sequence information provided by the human ESTs allowed the rapid cloning of cDNAs encoding human SOCS1.

The mouse and rat SOCS1 gene encodes a 212 amino acid protein whereas the human SOCS1 gene encodes a 211 amino acid protein. Mouse, rat and human SOCS1 proteins share 95-99% amino acid identity (Figure 9). A search of translated nucleic acid databases with the predicted amino acid sequence of SOCS1 showed that it was most related to a recently cloned cytokine-inducible immediate early gene product, CIS, and two classes of ESTs. Full length cDNAs from the two classes of ESTs were isolated and found to encode proteins of similar length and overall structure to SOCS1 and CIS. These clones were given the names SOCS2 and SOCS3. Each of the four proteins contains a central SH2 domain and a C-terminal region termed the SOCS motif. The SOCS1 proteins exhibit an extremely high level of amino acid sequence similarity (95-99% identity) amongst different species. However, the forms of the SOCS1, SOCS2, SOCS3 and CIS from the same animal, while

clearly defining a new family of SH2-containing proteins, exhibited a lower amino acid identity. SOCS2 and CIS exhibit approximately 38% amino acid identity, while the remaining members of the family share approximately 25% amino acid identity (Figure 9). The coding region of the genes for SOCS1 and SOC3 appear to contain no introns while the coding region of the genes for SOCS2 and CIS contain one and two introns, respectively.

The Genbank Acession Numbers for the sequences referred to herein are mouse SOCS1 cDNA (U88325), human SOCS1 cDNA (U88326), mouse SOCS2 cDNA (U88327), mouse SOCS3 cDNA (U88328).

EXAMPLE 13

CONSTITUTIVE EXPRESSION OF SOCS1 SUPPRESSES THE ACTION OF A RANGE OF CYTOKINES

To formally establish that the phenotype of the 4A2 cell line was directly related to expression of SOCS1, and not to unrelated genetic changes which may have occurred independently in these cells, a cDNA encoding an epitope-tagged version of SOCS1 under the control of the EF1α promoter was transfected into parental M1 cells, and M1 cells expressing the receptor for thrombopoietin, c-mpl (M1.mpl). Transfection of the SOCS1 expression vector into both cell lines resulted in an increase in the frequency of IL-6 unresponsive M1 cells.

Multiple independent clones of M1 cells expression SOCS1, as detected by Western blot, displayed a cytokine-unresponsive phenotype that was indistinguishable from 4A2. Further, if transfectants were not maintained in puromycin, expression of SOCS1 was lost over time and cells regained their cytokine responsiveness. In the absence of cytokine, colonies derived from 4A2 and other SOCS1 expressing clones characteristically grew to a smaller size than colones formed by control M1 cells (Figure 10).

The effect of constitutive SOCS1 expression on the response of M1 cells to a range of cytokines was investigated using the 4A2 cell line and a clone of M1.mpl cells expressing

SOCS1 (M1.mpl.SOCS1). Unlike parental M1 cells and M1.mpl cells, the two cell lines expressing SOCS1 continued to proliferate and failed to form differentiated colonies in response to either IL-6, LIF, OSM, IFN-γ or, in the case of the M1.mpl.SOCS1 cell line, thrombopoietin (Figure 4). For both cell lines, however, a normal response to dexamethasone was observed, suggesting that SOCS1 specifically affected cytokine signal transduction rather than differentiation *per se*. Consistent with these data, while parental M1 cells and M1.mpl cells became large and vacuolated in response to IL-6, 4A2 and M1.mpl.SOCS1 cells showed no evidence of morphological differentiation in response to IL-6 or other cytokines (Figure 5).

EXAMPLE 14

SOCS1 INHIBITS A RANGE OF IL-6 SIGNAL TRANSDUCTION PROCESSES, INCLUDING STAT3 PHOSPHORYLATION AND ACTIVATION

Phosphorylation of the cell surface receptor component gp130, the cytoplasmic tyrosine kinase JAK1 and the transcription factor STAT3 is thought to play a central role in IL-6 signal transduction. These events were compared in the parental M1 and M1.mpl cell lines and their SOCS1-expressing counterparts. As expected, gp130 was phosphorylated rapidly in response to IL-6 in both parental lines, however, this was reduced five- to ten-fold in the cell lines expressing SOCS1 (Figure 6). Likewise, STAT3 phosphorylation was also reduced by approximately ten-fold in response to IL-6 in those cell lines expressing SOCS1 (Figure 6). Consistent with a reduction in STAT3 phosphorylation, activation of specific STAT DNA binding complexes, as determined by electrophoretic mobility shift assay, was also reduced. Notably, there was a reduction in the formation of SIF-A (containing STAT3), SIF-B (STAT1/STAT3 heterodimer) and SIF-C (containing STAT1), the three STAT complexes induced in M1 cells stimulated with IL-6 (Figure 7). Similarly, constitutive expression of SOCS1 also inhibited IFN-γ-stimulated formation of p91 homodimers (Figure 7). STAT phosphorylation and activation were not the only cytoplasmic processes to be effected by SOCS1 expression, as the phosphorylation of other proteins, including shc and MAP kinase, was reduced to a similar extent (Figure 7).

EXAMPLE 15

TRANSCRIPTION OF THE SOCS1 GENE IS STIMULATED BY IL-6 *IN VITRO* AND *IN VIVO*

Although SOCS1 can inhibit cytokine signal transduction when constitutively expressed in M1 cells, this does not necessarily indicate that SOCS1 normally functions to negatively regulate an IL-6 response. In order to investigate this possibility the inventors determined whether transcription of the SOCS1 gene is regulated in the response of M1 cells to IL-6 and, because of the critical role IL-6 plays in regulating the acute phase response to injury and infection, the response of the liver to intravenous injection of 5 mg IL-6. In the absence of IL-6, SOCS1 mRNA was undetectable in either M1 cells or in the liver. However, for both cell types, a 1.4 kb SOCS1 transcript was induced within 20 to 40 minutes by IL-6 (Figure 8). For M1 cells, where the IL-6 was present throughout the experiment, the level of SOCS1 mRNA remained elevated (Figure 8). In contrast, IL-6 was administered in vivo by a single intravenous injection and was rapidly cleared from the circulation, resulting in a pulse of IL-6 stimulation to the liver. Consistent with this, transient expression of SOCS1 mRNA was detectable in the liver, peaking approximately 40 minutes after injection and declining to basal levels within 4 hours (Figure 8).

EXAMPLE 16

REGULATION OF SOCS GENES

Since CIS was cloned as a cytokine-inducible immediate early gene the inventors examined whether SOCS1, SOCS2 and SOCS3 were similarly regulated. The basal pattern of expression of the four SOCS genes was examined by Northern blot analysis of mRNA from a variety of tissues from male and female C57B1/6 mice (Figure 11A). Constitutive expression of SOCS1 was observed in the thymus and to a lesser extend in the spleen and the lung. SOCS2 expression was restricted primarily to the testis and in some animals the liver and lung; for SOCS3 a low level of expression was observed in the lung, spleen and thymus, while CIS expression was more widespread, including the testis, heart, lung, kidney and, in some animals, the liver.

The inventors sought to determine whether expression of the four SOCS genes was regulated by IL-6. Northern blots of mRNA prepared from the livers of untreated and IL-6-injected mice, or from unstimulated and IL-6-stimulated M1 cells, were hybridised with labelled fragments of SOCS1, SOCS2, SOCS3 and CIS cDNAs (FiGure 11B). Expression of all four SOCS genes was increased in the liver following IL-6 injection, however the kinetics of induction appeared to differ. Expression of SOCS1 and SOCS3 was transient in the liver, with mRNA detectable after 20 minutes of IL-6 injection and declining to basal levels within 4 hours for SOCS and 8 hours for SOCS3. Induction of SOCS2 and CIS mRNA in the liver followed similar initial kinetics to that of SOCS1, but was maintained at an elevated level for at least 24 hours. A similar induction of SOCS gene mRNA was observed in other organs, notably the lung and the spleen. In contrast, in M1 cells, while SOCS1 and CIS mRNA were induced by IL-6, no induction of either SOCS2 or SOCS3 expression was detected. This result highlights cell type-specific differences in the expression of the genes of SOCS family members in response to the same cytokine.

In order to examine the spectrum of cytokines that was capable of inducing transcription of the various members of the SOCS gene family, bone marrow cells were stimulated for an hour with a range of cytokines, after which mRNA was extracted and cDNA was synthesised. PCR was then used to assess the expression of SOCS1, SOCS2, SOCS3 and CIS (Figure 11C). In the absence of stimulation, little or no expression of any of the SOCS genes was detectable in bone marrow by PCR. Stimulation of bone marrow cells with a broad array of cytokines appeared capable of up regulating mRNA for one or more members of the SOCS family. IFNγ, for example, induced expression of all four SOCS genes, while erythropoietin, granulocyte colony-stimulating factor, granulocyte-macrophage colony stimulating factor and interleukin-3 induced expression of SOCS2, SOCS3 and CIS. Interestingly, tumor necrosis factor alpha, macrophage colony-stimulating factor and interleukin-1, which act through receptors that do not fall into the type I cytokine receptor class also appeared capable of inducing expression of SOCS3 and CIS, suggesting that SOCS proteins may play a broader role in regulating signal transduction.

As constitutive expression of SOCS1 inhibited the response of M1 cells to a range of cytokines, the inventors examined whether phosphorylation of the cell surface receptor component gp130 and the transcription factor STA3, which are though to play a central role in IL-6 signal transduction, were affected. These events were compared in the parental M1 and M1.mpl cell lines and their SOCS1-expressing counterparts. As expected, gp130 was phyosphorylated rapidly in response to IL-6 in both parental lines, however, this was reduced in the cell lines expressing SOCS1 (Figure 12A). Likewise, STAT3 phosphorylation was also reduced in response to IL-6 in those cell lines expressing SOCS1 (Figure 12A). Consistent with a reduction in STAT3 phosphorylation, activation of specific STA/DNA binding complexes, as determined by electrophoretic mobility shift assay, was also reduced. Notably, there was a failure to form SIF-A (containing STAT3) and SIF-B(STAT1/STAT3 heterodimer), the major STAT complexes induced in M1 cells stimulated with IL-6 (Figure 12B). Similarly, constitutive expression of SOCS1 also inhibited IFNγ-stimulating formation of SIF-C (STAT1 homodimer; Figure 12B). These experiments are consistent with the proposal that SOCS1 inhibits signal transduction upstream of receptor and STAT phosphorylation, potentially at the level of the JAK kinases.

The ability of SOCS1 to inhibit signal transduction and ultimately the biological response to cytokines suggest that, like the SH2-containing phosphatase SHP-1 (Ihle *et al,* 1994; Yi *et al,* 1993), the SOCS proteins may play a central role in controlling the intensity and/or duration of a cell's response to a diverse range of extracellular stimuli by suppressing the signal transduction process. The evidence provided here indicates that the SOCS family acts in a classical negative feedback loop for cytokine signal transduction. Like other genes such as OSM, expression of genes encoding the SOCS proteins is induced by cytokines through the activation of STATs. Once expressed, it is proposed that the SOCS proteins inhibit the activity of JAKs and so reduce the phosphorylation of receptors and STATs, thereby suppressing signal transduction and any ensuing biological response. Importantly, inhibition of STAT activation will, over time, lead to a reduction in SOCS gene expression, allowing cells to regain responsiveness to cytokines.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CACGCCGCCC ACGTGAAGGC 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTCGCCAATG ACAAGACGCT 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1236 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..636

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGAGGCTCAA GCTCCGGGCG GATTCTGCGT GCCGCTCTCG CTCCTTGGGG TCTGTTGGCC -101

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GACGCTATGG CCCACCCCTC CAGCTGGCCC CTCGAGTAGG                           -1


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  1               5                  10                  15

GCA GAG CCC CGA CGG CGG TCA GAG CCC TCC TCG TCC TCG TCT TCG TCC        96
Ala Glu Pro Arg Arg Arg Ser Glu Pro Ser Ser Ser Ser Ser Ser Ser
            20                  25                  30

TCG CCA GCG GCC CCC GTG CGT CCC CGG CCC TGC CCG GCG GTC CCA GCC       144
Ser Pro Ala Ala Pro Val Arg Pro Arg Pro Cys Pro Ala Val Pro Ala
        35                  40                  45

CCA GCC CCT GGC GAC ACT CAC TTC CGC ACC TTC CGC TCC CAC TCC GAT       192
Pro Ala Pro Gly Asp Thr His Phe Arg Thr Phe Arg Ser His Ser Asp
    50                  55                  60

TAC CGG CGC ATC ACG CGG ACC AGC GCG CTC CTG GAC GCC TGC GGC TTC       240
Tyr Arg Arg Ile Thr Arg Thr Ser Ala Leu Leu Asp Ala Cys Gly Phe
65                  70                  75                  80

TAT TGG GGA CCC CTG AGC GTG CAC GGG GCG CAC GAG CGG CTG CGT GCC       288
Tyr Trp Gly Pro Leu Ser Val His Gly Ala His Glu Arg Leu Arg Ala
                85                  90                  95

GAG CCC GTG GGC ACC TTC TTG GTG CGC GAC AGT CGT CAA CGG AAC TGC       336
Glu Pro Val Gly Thr Phe Leu Val Arg Asp Ser Arg Gln Arg Asn Cys
            100                 105                 110

TTC TTC GCG CTC AGC GTG AAG ATG GCT TCG GGC CCC ACG AGC ATC CGC       384
Phe Phe Ala Leu Ser Val Lys Met Ala Ser Gly Pro Thr Ser Ile Arg
        115                 120                 125

GTG CAC TTC CAG GCC GGC CGC TTC CAC TTG GAC GGC AGC CGC GAG ACC       432
Val His Phe Gln Ala Gly Arg Phe His Leu Asp Gly Ser Arg Glu Thr
    130                 135                 140

TTC GAC TGC CTT TTC GAG CTG CTG GAG CAC TAC GTG GCG GCG CCG CGC       480
Phe Asp Cys Leu Phe Glu Leu Leu Glu His Tyr Val Ala Ala Pro Arg
145                 150                 155                 160

CGC ATG TTG GGG GCC CCG CTG CGC CAG CGC CGC GTG CGG CCG CTG CAG       528
Arg Met Leu Gly Ala Pro Leu Arg Gln Arg Arg Val Arg Pro Leu Gln
                165                 170                 175

GAG CTG TGT CGC CAG CGC ATC GTG GCC GCC GTG GGT CGC GAG AAC CTG       576
Glu Leu Cys Arg Gln Arg Ile Val Ala Ala Val Gly Arg Glu Asn Leu
            180                 185                 190

GCG CGC ATC CCT CTT AAC CCG GTA CTC CGT GAC TAC CTG AGT TCC TTC       624
Ala Arg Ile Pro Leu Asn Pro Val Leu Arg Asp Tyr Leu Ser Ser Phe
```

```
        195                 200                 205

CCC TTC CAG ATC TGA CCGGCTG CCGCTGTGCC GCAGCATTAA GTGGGGGCGC          676
Pro Phe Gln Ile  *
    210

CTTATTATTT CTTATTATTA ATTATTATTA TTTTTCTGGA ACCACGTGGG AGCCCTCCCC     736

GCCTGGGTCG GAGGGAGTGG TTGTGGAGGG TGAGATGCCT CCCACTTCTG GCTGGAGACC     796

TCATCCCACC TCTCAGGGGT GGGGGTGCTC CCCTCCTGGT GCTCCCTCCG GGTCCCCCCT     856

GGTTGTAGCA GCTTGTGTCT GGGGCCAGGA CCTGAATTCC ACTCCTACCT CTCCATGTTT     916

ACATATTCCC AGTATCTTTG CACAAACCAG GGGTCGGGGA GGGTCTCTGG CTTCATTTTT     976

CTGCTGTGCA GAATATCCTA TTTTATATTT TTACAGCCAG TTTAGGTAAT AAACTTTATT    1036

ATGAAAGTTT TTTTTTAAAA GAAAAAAAAA AAAAAAAAA                          1075
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 212 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Val Ala Arg Asn Gln Val Ala Ala Asp Asn Ala Ile Ser Pro Ala
 1               5                  10                  15

Ala Glu Pro Arg Arg Arg Ser Glu Pro Ser Ser Ser Ser Ser Ser Ser
            20                  25                  30

Ser Pro Ala Ala Pro Val Arg Pro Arg Pro Cys Pro Ala Val Pro Ala
        35                  40                  45

Pro Ala Pro Gly Asp Thr His Phe Arg Thr Phe Arg Ser His Ser Asp
    50                  55                  60

Tyr Arg Arg Ile Thr Arg Thr Ser Ala Leu Leu Asp Ala Cys Gly Phe
65                  70                  75                  80

Tyr Trp Gly Pro Leu Ser Val His Gly Ala His Glu Arg Leu Arg Ala
                85                  90                  95

Glu Pro Val Gly Thr Phe Leu Val Arg Asp Ser Arg Gln Arg Asn Cys
            100                 105                 110
```

```
Phe Phe Ala Leu Ser Val Lys Met Ala Ser Gly Pro Thr Ser Ile Arg
        115                 120                 125

Val His Phe Gln Ala Gly Arg Phe His Leu Asp Gly Ser Arg Glu Thr
    130                 135                 140

Phe Asp Cys Leu Phe Glu Leu Leu Glu His Tyr Val Ala Ala Pro Arg
145                 150                 155                 160

Arg Met Leu Gly Ala Pro Leu Arg Gln Arg Arg Val Arg Pro Leu Gln
                165                 170                 175

Glu Leu Cys Arg Gln Arg Ile Val Ala Ala Val Gly Arg Glu Asn Leu
            180                 185                 190

Ala Arg Ile Pro Leu Asn Pro Val Leu Arg Asp Tyr Leu Ser Ser Phe
        195                 200                 205

Pro Phe Gln Ile
    210
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1121 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 223..819

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
GCGATCTGTG GGTGACAGTG TCTGCGAGAG ACTTTGCCAC ACCATTCTGC CGGAATTTGG      60

AGAAAAAGAA CCAGCCGCTT CCAGTCCCCT CCCCCTCCGC CACCATTTCG GACACCCTGC     120

ACACTCTCGT TTTGGGGTAC CCTGTGACTT CCAGGCAGCA CGCGAGGTCC ACTGGCCCCA     180

GCTCGGGCGA CCAGCTGTCT GGGACGTGTT GACTCATCTC CC ATG ACC CTG CGG         234
                                                 Met Thr Leu Arg
                                                   1

TGC CTG GAG CCC TCC GGG AAT GGA GCG GAC AGG ACG CGG AGC CAG TGG         282
Cys Leu Glu Pro Ser Gly Asn Gly Ala Asp Arg Thr Arg Ser Gln Trp
  5                 10                  15                  20
```

```
GGG ACC GCG GGG TTG CCG GAG GAA CAG TCC CCC GAG GCG GCG CGT CTG        330
Gly Thr Ala Gly Leu Pro Glu Glu Gln Ser Pro Glu Ala Ala Arg Leu
                  25                  30                  35

GCG AAA GCC CTG CGC GAG CTC AGT CAA ACA GGA TGG TAC TGG GGA AGT        378
Ala Lys Ala Leu Arg Glu Leu Ser Gln Thr Gly Trp Tyr Trp Gly Ser
              40                  45                  50

ATG ACT GTT AAT GAA GCC AAA GAG AAA TTA AAA GAG GCT CCA GAA GGA        426
Met Thr Val Asn Glu Ala Lys Glu Lys Leu Lys Glu Ala Pro Glu Gly
          55                  60                  65

ACT TTC TTG ATT AGA GAT AGT TCG CAT TCA GAC TAC CTA CTA ACT ATA        474
Thr Phe Leu Ile Arg Asp Ser Ser His Ser Asp Tyr Leu Leu Thr Ile
      70                  75                  80

TCC GTT AAG ACG TCA GCT GGA CCG ACT AAC CTG CGG ATT GAG TAC CAA        522
Ser Val Lys Thr Ser Ala Gly Pro Thr Asn Leu Arg Ile Glu Tyr Gln
  85                  90                  95                 100

GAT GGG AAA TTC AGA TTG GAT TCT ATC ATA TGT GTC AAG TCC AAG CTT        570
Asp Gly Lys Phe Arg Leu Asp Ser Ile Ile Cys Val Lys Ser Lys Leu
                 105                 110                 115

AAA CAG TTT GAC AGT GTG GTT CAT CTG ATT GAC TAC TAT GTC CAG ATG        618
Lys Gln Phe Asp Ser Val Val His Leu Ile Asp Tyr Tyr Val Gln Met
             120                 125                 130

TGC AAG GAT AAA CGG ACA GGC CCA GAA GCC CCA CGG AAT GGG ACT GTT        666
Cys Lys Asp Lys Arg Thr Gly Pro Glu Ala Pro Arg Asn Gly Thr Val
         135                 140                 145

CAC CTG TAC CTG ACC AAA CCT CTG TAT ACA TCA GCA CCC ACT CTG CAG        714
His Leu Tyr Leu Thr Lys Pro Leu Tyr Thr Ser Ala Pro Thr Leu Gln
     150                 155                 160

CAT TTC TGT CGA CTC GCC ATT AAC AAA TGT ACC GGT ACG ATC TGG GGA        762
His Phe Cys Arg Leu Ala Ile Asn Lys Cys Thr Gly Thr Ile Trp Gly
165                 170                 175                 180

CTG CCT TTA CCA ACA AGA CTA AAA GAT TAC TTG GAA GAA TAT AAA TTC        810
Leu Pro Leu Pro Thr Arg Leu Lys Asp Tyr Leu Glu Glu Tyr Lys Phe
                 185                 190                 195

CAG GTA TAAGTATTTC TCTCTCTTTT TCGTTTTTTT TTAAAAAAAA AAAAACACAT        866
Gln Val

GCCTCATATA GACTATCTCC GAATGCAGCT ATGTGAAAGA GAACCCAGAG GCCCTCCTCT     926

GGATAACTGC GCAGAATTCT CTCTTAAGGA CAGTTGGGCT CAGTCTAACT TAAAGGTGTG     986

AAGATGTAGC TAGGTATTTT AAAGTTCCCC TTAGGTAGTT TTAGCTGAAT GATGCTTTCT    1046

TTCCTATGGC TGCTCAAGAT CAAATGGCCC TTTTAAATGA AACAAAACAA AACAAAACAA    1106
```

AAAAAAAAAA AAAAA 1121

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 198 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Thr Leu Arg Cys Leu Glu Pro Ser Gly Asn Gly Ala Asp Arg Thr
 1               5                  10                  15

Arg Ser Gln Trp Gly Thr Ala Gly Leu Pro Glu Glu Gln Ser Pro Glu
            20                  25                  30

Ala Ala Arg Leu Ala Lys Ala Leu Arg Glu Leu Ser Gln Thr Gly Trp
        35                  40                  45

Tyr Trp Gly Ser Met Thr Val Asn Glu Ala Lys Glu Lys Leu Lys Glu
    50                  55                  60

Ala Pro Glu Gly Thr Phe Leu Ile Arg Asp Ser Ser His Ser Asp Tyr
65                  70                  75                  80

Leu Leu Thr Ile Ser Val Lys Thr Ser Ala Gly Pro Thr Asn Leu Arg
                85                  90                  95

Ile Glu Tyr Gln Asp Gly Lys Phe Arg Leu Asp Ser Ile Ile Cys Val
            100                 105                 110

Lys Ser Lys Leu Lys Gln Phe Asp Ser Val Val His Leu Ile Asp Tyr
        115                 120                 125

Tyr Val Gln Met Cys Lys Asp Lys Arg Thr Gly Pro Glu Ala Pro Arg
    130                 135                 140

Asn Gly Thr Val His Leu Tyr Leu Thr Lys Pro Leu Tyr Thr Ser Ala
145                 150                 155                 160

Pro Thr Leu Gln His Phe Cys Arg Leu Ala Ile Asn Lys Cys Thr Gly
                165                 170                 175

Thr Ile Trp Gly Leu Pro Leu Pro Thr Arg Leu Lys Asp Tyr Leu Glu
            180                 185                 190

Glu Tyr Lys Phe Gln Val
        195
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2187 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 18..695

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CGCTGGCTCC GTGCGCC ATG GTC ACC CAC AGC AAG TTT CCC GCC GCC GGG          50
                   Met Val Thr His Ser Lys Phe Pro Ala Ala Gly
                     1               5                  10

ATG AGC CGC CCC CTG GAC ACC AGC CTG CGC CTC AAG ACC TTC AGC TCC          98
Met Ser Arg Pro Leu Asp Thr Ser Leu Arg Leu Lys Thr Phe Ser Ser
             15                  20                  25

AAA AGC GAG TAC CAG CTG GTG GTG AAC GCC GTG CGC AAG CTG CAG GAG         146
Lys Ser Glu Tyr Gln Leu Val Val Asn Ala Val Arg Lys Leu Gln Glu
         30                  35                  40

AGC GGA TTC TAC TGG AGC GCC GTG ACC GGC GGC GAG GCG AAC CTG CTG         194
Ser Gly Phe Tyr Trp Ser Ala Val Thr Gly Gly Glu Ala Asn Leu Leu
     45                  50                  55

CTC AGC GCC GAG CCC GCG GGC ACC TTT CTT ATC CGC GAC AGC TCG GAC         242
Leu Ser Ala Glu Pro Ala Gly Thr Phe Leu Ile Arg Asp Ser Ser Asp
 60                  65                  70                  75

CAG CGC CAC TTC TTC ACG TTG AGC GTC AAG ACC CAG TCG GGG ACC AAG         290
Gln Arg His Phe Phe Thr Leu Ser Val Lys Thr Gln Ser Gly Thr Lys
                 80                  85                  90

AAC CTA CGC ATC CAG TGT GAG GGG GGC AGC TTT TCG CTG CAG AGT GAC         338
Asn Leu Arg Ile Gln Cys Glu Gly Gly Ser Phe Ser Leu Gln Ser Asp
             95                  100                 105

CCC CGA AGC ACG CAG CCA GTT CCC CGC TTC GAC TGT GTA CTC AAG CTG         386
Pro Arg Ser Thr Gln Pro Val Pro Arg Phe Asp Cys Val Leu Lys Leu
         110                 115                 120

GTG CAC CAC TAC ATG CCG CCT CCA GGG ACC CCC TCC TTT TCT TTG CCA         434
Val His His Tyr Met Pro Pro Pro Gly Thr Pro Ser Phe Ser Leu Pro
     125                 130                 135

CCC ACG GAA CCC TCG TCC GAA GTT CCG GAG CAG CCA CCT GCC CAG GCA         482
Pro Thr Glu Pro Ser Ser Glu Val Pro Glu Gln Pro Pro Ala Gln Ala
140                 145                 150                 155
```

```
CTC CCC GGG AGT ACC CCC AAG AGA GCT TAC TAC ATC TAT TCT GGG GGC        530
Leu Pro Gly Ser Thr Pro Lys Arg Ala Tyr Tyr Ile Tyr Ser Gly Gly
                160                 165                 170

GAG AAG ATT CCG CTG GTA CTG AGC CGA CCT CTC TCC TCC AAC GTG GCC        578
Glu Lys Ile Pro Leu Val Leu Ser Arg Pro Leu Ser Ser Asn Val Ala
            175                 180                 185

ACC CTC CAG CAT CTT TGT CGG AAG ACT GTC AAC GGC CAC CTG GAC TCC        626
Thr Leu Gln His Leu Cys Arg Lys Thr Val Asn Gly His Leu Asp Ser
        190                 195                 200

TAT GAG AAA GTG ACC CAG CTG CCT GGA CCC ATT CGG GAG TTC CTG GAT        674
Tyr Glu Lys Val Thr Gln Leu Pro Gly Pro Ile Arg Glu Phe Leu Asp
    205                 210                 215

CAG TAT GAT GCT CCA CTT TAAGGAGCAA AAGGGTCAGA GGGGGGCCTG               722
Gln Tyr Asp Ala Pro Leu
220                 225

GGTCGGTCGG TCGCCTCTCC TCCGAGGCAC ATGGCACAAG CACAAAAATC CAGCCCCAAC     782

GGTCGGTAGC TCCCAGTGAG CCAGGGGCAG ATTGGCTTCT TCCTCAGGCC CTCCACTCCC     842

GCAGAGTAGA GCTGGCAGGA CCTGGAATTC GTCTGAGGGG AGGGGGAGCT GCCACCTGCT     902

TTCCCCCCTC CCCCAGCTCC AGCTTCTTTC AAGTGGAGCC AGCCGGCCTG GCCTGGTGGG     962

ACAATACCTT TGACAAGCGG ACTCTCCCCT CCCCTTCCTC CACACCCCCT CTGCTTCCCA    1022

AGGGAGGTGG GGACACCTCC AAGTGTTGAA CTTAGAACTG CAAGGGGAAT CTTCAAACTT    1082

TCCCGCTGGA ACTTGTTTGC GCTTTGATTT GGTTTGATCA AGAGCAGGCA CCTGGGGGAA    1142

GGATGGAAGA GAAAAGGGTG TGTGAAGGGT TTTTATGCTG GCCAAAGAAA TAACCACTCC    1202

CACTGCCCAA CCTAGGTGAG GAGTGGTGGC TCCTGGCTCT GGGGAGAGTG GCAAGGGGTG    1262

ACCTGAAGAG AGCTATACTG GTGCCAGGCT CCTCTCCATG GGGCAGCTAA TGAAACCTCG    1322

CAGATCCCTT GCACCCCAGA ACCCTCCCCG TTGTGAAGAG GCAGTAGCAT TTAGAAGGGA    1382

GACAGATGAG GCTGGTGAGC TGGCCGCCTT TTCCAACACC GAAGGGAGGC AGATCAACAG    1442

ATGAGCCATC TTGGAGCCCA GGTTTCCCCT GGAGCAGATG GAGGGTTCTG CTTTGTCTCT    1502

CCTATGTGGG GCTAGGAGAC TCGCCTTAAA TGCCCTCTGT CCCAGGGATG GGGATTGGCA    1562

CACAAGGAGC CAAACACAGC CAATAGGCAG AGAGTTGAGG GATTCACCCA GGTGGCTACA    1622

GGCCAGGGGA AGTGGCTGCA GGGGAGAGAC CCAGTCACTC CAGGAGACTC CTGAGTTAAC    1682

ACTGGGAAGA CATTGGCCAG TCCTAGTCAT CTCTCGGTCA GTAGGTCCGA GAGCTTCCAG    1742

GCCCTGCACA GCCCTCCTTT CTCACCTGGG GGGAGGCAGG AGGTGATGGA GAAGCCTTCC    1802
```

CATGCCGCTC ACAGGGGCCT CACGGGAATG CAGCAGCCAT GCAATTACCT GGAACTGGTC 1862

CTGTGTTGGG GAGAAACAAG TTTTCTGAAG TCAGGTATGG GGCTGGGTGG GGCAGCTGTG 1922

TGTTGGGGTG GCTTTTTTCT CTCTGTTTTG AATAATGTTT ACAATTTGCC TCAATCACTT 1982

TTATAAAAAT CCACCTCCAG CCCGCCCCTC TCCCCACTCA GGCCTTCGAG GCTGTCTGAA 2042

GATGCTTGAA AAACTCAACC AAATCCCAGT TCAACTCAGA CTTTGCACAT ATATTTATAT 2102

TTATACTCAG AAAAGAAACA TTTCAGTAAT TTATAATAAA AGAGCACTAT TTTTTAATGA 2162

AAAAAAAAAA AAAAAAAAAA AAAAA 2187

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 225 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Val Thr His Ser Lys Phe Pro Ala Ala Gly Met Ser Arg Pro Leu
 1               5                  10                  15

Asp Thr Ser Leu Arg Leu Lys Thr Phe Ser Ser Lys Ser Glu Tyr Gln
            20                  25                  30

Leu Val Val Asn Ala Val Arg Lys Leu Gln Glu Ser Gly Phe Tyr Trp
        35                  40                  45

Ser Ala Val Thr Gly Gly Glu Ala Asn Leu Leu Leu Ser Ala Glu Pro
    50                  55                  60

Ala Gly Thr Phe Leu Ile Arg Asp Ser Ser Asp Gln Arg His Phe Phe
65                  70                  75                  80

Thr Leu Ser Val Lys Thr Gln Ser Gly Thr Lys Asn Leu Arg Ile Gln
                85                  90                  95

Cys Glu Gly Gly Ser Phe Ser Leu Gln Ser Asp Pro Arg Ser Thr Gln
            100                 105                 110

Pro Val Pro Arg Phe Asp Cys Val Leu Lys Leu Val His His Tyr Met
        115                 120                 125

Pro Pro Pro Gly Thr Pro Ser Phe Ser Leu Pro Pro Thr Glu Pro Ser
    130                 135                 140

Ser Glu Val Pro Glu Gln Pro Pro Ala Gln Ala Leu Pro Gly Ser Thr
145                 150                 155                 160
```

Pro Lys Arg Ala Tyr Tyr Ile Tyr Ser Gly Gly Glu Lys Ile Pro Leu
165 170 175

Val Leu Ser Arg Pro Leu Ser Ser Asn Val Ala Thr Leu Gln His Leu
180 185 190

Cys Arg Lys Thr Val Asn Gly His Leu Asp Ser Tyr Glu Lys Val Thr
195 200 205

Gln Leu Pro Gly Pro Ile Arg Glu Phe Leu Asp Gln Tyr Asp Ala Pro
210 215 220

Leu
225

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1094 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTCCGGCTGG CCCCTTCTGT AGGATGGTAG CACACAACCA GGTGGCAGCC GACAATGCAG 60
TCTCCACAGC AGCAGAGCCC CGACGGCGGC CAGAACCTTC CTCCTCTTCC TCCTCCTCGC 120
CCGCGGCCCC CGCGCGCCCG CGGCCGTGCC CCGCGGTCCC GGCCCCGGCC CCCGGCGACA 180
CGCACTTCCG CACATTCCGT TCGCACGCCG ATTACCGGCG CATCACGCGC GCCAGCGCGC 240
TCCTGGACGC CTGCGGATTC TACTGGGGGC CCCTGAGCGT GCACGGGGCG CACGAGCGGC 300
TGCGCGCCGA GCCCGTGGGC ACCTTCCTGG TGCGCGACAG CCGCCAGCGG AACTGCTTTT 360
TCGCCCTTAG CGTGAAGATG GCCTCGGGAC CCACGAGCAT CCGCGTGCAC TTTCAGGCCG 420
GCCGCTTTCA CCTGGATGGC AGCCGCGAGA GCTTCGACTG CCTCTTCGAG CTGCTGGAGC 480
ACTACGTGGC GGCGCCGCGC CGCATGCTGG GGGCCCCGCT GCGCCAGCGC CGCGTGCGGC 540
CGCTGCAGGA GCTGTGCCGC CAGCGCATCG TGGCCACCGT GGGCCGCGAG AACCTGGCTC 600
GCATCCCCCT CAACCCCGTC CTCCGCGACT ACCTGAGCTC CTTCCCCTTC CAGATTTGAC 660
CGGCAGCGCC CGCCGTGCAC GCAGCATTAA CTGGGATGCC GTGTTATTTT GTTATTACTT 720
GCCTGGAACC ATGTGGGTAC CCTCCCCGGC CTGGGTTGGA GGGAGCGGAT GGGTGTAGGG 780
GCGAGGCGCC TCCCGCCCTC GGCTGGAGAC GAGGCCGCAG ACCCCTTCTC ACCTCTTGAG 840
GGGGTCCTCC CCCTCCTGGT GCTCCCTCTG GGTCCCCCTG GTTGTTGTAG CAGCTTAACT 900
GTATCTGGAG CCAGGACCTG AACTCGCACC TCCTACCTCT TCATGTTTAC ATATACCCAG 960
TATCTTTGCA CAAACCAGGG GTTGGGGGAG GGTCTCTGGC TTTATTTTTC TGCTGTGCAG 1020

AATCCTATTT TATATTTTTT AAAGTCAGTT TAGGTAATAA ACTTTATTAT GAAAGTTTTT 1080

TTTTTTAAAA AAAA 1094

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 211 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Val Ala His Asn Gln Val Ala Ala Asp Asn Ala Val Ser Thr Ala
1               5                  10                  15

Ala Glu Pro Arg Arg Arg Pro Glu Pro Ser Ser Ser Ser Ser Ser Ser
            20                  25                  30

Pro Ala Ala Pro Ala Arg Pro Arg Pro Cys Pro Ala Val Pro Ala Pro
        35                  40                  45

Ala Pro Gly Asp Thr His Phe Arg Thr Phe Arg Ser His Ala Asp Tyr
    50                  55                  60

Arg Arg Ile Thr Arg Ala Ser Ala Leu Leu Asp Ala Cys Gly Phe Tyr
65                  70                  75                  80

Trp Gly Pro Leu Ser Val His Gly Ala His Glu Arg Leu Arg Ala Glu
                85                  90                  95

Pro Val Gly Thr Phe Leu Val Arg Asp Ser Arg Gln Arg Asn Cys Phe
```

100 105 110

Phe Ala Leu Ser Val Lys Met Ala Ser Gly Pro Thr Ser Ile Arg Val
115 120 125

His Phe Gln Ala Gly Arg Phe His Leu Asp Gly Ser Arg Glu Ser Phe
130 135 140

Asp Cys Leu Phe Glu Leu Leu Glu His Tyr Val Ala Ala Pro Arg Arg
145 150 155 160

Met Leu Gly Ala Pro Leu Arg Gln Arg Arg Val Arg Pro Leu Gln Glu
165 170 175

Leu Cys Arg Gln Arg Ile Val Ala Thr Val Gly Arg Glu Asn Leu Ala
180 185 190

Arg Ile Pro Leu Asn Pro Val Leu Arg Asp Tyr Leu Ser Ser Phe Pro
195 200 205

Phe Gln Ile
210

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2807 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGAAACCGAG GCGGGGAGAC CAGGAGGCCT TGGCCTCAGA GCTTCAGAGT CGCGTGGCAG 60
CAAACAGAGA AACCTGTAGA GGGCAGTGTG CGTCACTTAG CTCAGGGAAG CTGCACGCGA 120

AACTCACCCG CCTTCATTCA TAAACATCGT CAGCTAGGCA CCTACTCCTG GGCTTTCAGG 180
ACAAACTGAA TCACGAAACC ACAGTGTCCT TAAAATAGGT CTGACCGCCT GAATCCCTGG 240
CCAAGGTGTG TACGGGGCAT GGGAGCCCTT GTGCAGAGAT GCTTGCAGGA GCCTTGAGGG 300
GCTCTGTAAG ACAGAGGCTA GGAAGACAAA GTTGGGGGCT ACAGCTTCTT GTCCTGCCCG 360
GGGCCTCAGT TTCTTCGGTT GCCCACGTAG GAGTGCAGAG AGTCCAGCCC CTGGGGACCC 420
AACCCAACCC CGCCCAGTTT CCGAGGAACT CGTCCGGGAG CGGGGGCGCC CCTCCCGCAC 480
CGCCTTAGGC TTCCTTTGAA GCCTCTGCGG TCAGGCCACC GCTTCCTGGG AAGCCCAAGC 540
CAAGGCCAGG CCGAGTGGCC AACGGGAGGG GCCCGCGCGC GATTCTGGAG GAGGGCGGCG 600
GCCCCACAGG TCTCCAGGGC TGGCTAGCCG GGCTCCTAGA GCGGAGACTG CCAAGGCCTT 660
CGGGTCCTGG GCAGGAAGGA TCCTGGCAGG GAGGAGTTGC TTGGGGGGTG GGGGGGAAAG 720
GCTCCAGGCG CGGTGGAGCT CTGACCAGGA GAATGCACAC ACTCGGAGGG GAGGAGGCGT 780
GTCAGCCCCA AGCTAGCATC CCACCCGGGG AGCAGCGATG TGGGGCGAAG GTAGCCAGAG 840
CAAAAGAGCA GGCACCAGGT GACACGAAAC AGAAGATTCC GGGTAGAGCC AGAACCCCAG 900
AAGTCCCATT CAGGGAAGGT GCGAGGCGAG AACGAGTTAG GTGGACCCTC TCCAGGGGCA 960
GCCAAAGAAA TCTAAAGAGA ACCCGAAGGA CTTGCCGGAA AGAGAAACCG AAAGCGGCGG 1020
TGGGCGGGAT CGGTGGGCGG GGCCTCCCTG GTTTAAGAGC TTGATGCAGG GGCGGGCAGC 1080
AGCAGAGAGA ACTGCGGCCG TGGCAGCGGC ACGGCTCCCG GCCCCGGAGC ATGCGCGACA 1140
GCAGCCCCGG AACCCCCAGC CGCGGCGCCC CGCGTCCCGC CGCCAGGTGA GCCGAGGCAG 1200
CTGCGAAGGA GCAGGCGGGA GGGGATGGGA GGAAGGGGAG CAGAGCCTGG CAGGACTATC 1260
CTCGCAGACT GCATGGCGGG GTCGTGGATG CTATGCCTCT GGCGCCCGCC CCACCGGCTG 1320
GCCCAGGCGG CCCCTCGCGC GCGCGGGGCG CCGTCAGCCC CTCCTCTCCG GCCCTGAGCC 1380
CGGATCGTCC GCCCGGGTTC CAGTTCCCGG CGTGGCCAGT AGGCGGCAAC CGCGAGGCGG 1440
CAAGCCACCC AGCGGGGACG GCCTGGAGTC GGGCCCCTCT CCACGCCCCC TTCTCCACGC 1500
GCGCGGGGAG GCAGGGCTCC ACCGCCAGTC TGGAAGGGTT CCACATACAG GAACGGCCTA 1560
CTTCGCAGAT GAGCCCACCG AGGCTCAGGC TCCGGGCGGA TTCTGCGTGT CACCCTCGCT 1620
CCTTGGGGTC CGCTGGCCGG CCTGTGCCAC CCGGACGCCC GGTTCACTGC CTCTGTCTCC 1680
CCCATCAGCG CAGCCCCGGA CGCTATGGCC CACCCCTCCA GCTGGCCCCT CGAGTAGGAT 1740
GGTAGCACGT AACCAGGTGG AAGCCGACAA TGCGATCTCC CCGGCATCAG AGCCCCGACG 1800
GCGGCCAGAG CCATCCTCGT CCTCGTCTTC GTCCTCGCCG GCGGCCCCGG CGCGTCCCCG 1860
GCCCTGCCCG GTGGTCCCGG CCCCGGCTCC GGGCGACACT CACTTCCGCA CCTTCCGCTC 1920
CCACTCTGAT TACCGGCGCA TCACGCGGAC CAGCGCTCTC CTGGACGCCT GCGGCTTCTA 1980
CTGGGGACCC CTGAGCGTGC ATGGGGCGCA CGAACGGCTG CGTTCCGAAC CCGTGGGCAC 2040
CTTCTTGGTG CGCGACAGTC GCCAGCGGAA CTGCTTCTTC GCGCTCAGCG TGAAGATGGC 2100
TTCGGGCCCC ACGAGCATTC GTGTGCACTT CCAGGCCGGC CGCTTCCACC TGGACGGCAA 2160
CCGCGAGACC TTCGACTGCC TCTTCGAGCT GCTGGAGCAC TACGTGGCGG CGCCGCGCCG 2220
CATGTTGGGG GCCCCACTGC GCCAGCGCCG CGTGCGGCCG CTGCAGGAGC TGTGTCGCCA 2280
GCGCATCGTG GCCGCCGTGG GTCGCGAGAA CCTGGCACGC ATCCCTCTTA ACCCGGTACT 2340

CCGTGACTAC CTGAGTTCCT TCCCCTTCCA GATCTGACCG GCTGCCGCCG TGCCCGCAGA 2400
ATTAAGTGGG AGCGCCTTAT TATTTCTTAT TATTAATTAT TATTATTTTT CTGGAACCAC 2460
GTGGGAGCCC TCCCCGCCTA GGTCGGAGGG AGTGGGTGTG GAGGGTGAGA TCCCTCCCAC 2520
TTCTGGCTGG AGACCTTATC CCGCCTCTCG GGGGGCCTCC CCTCCTGGTG CTCCCTCCCG 2580
GTCCCCCTGG TTGTAGCAGC TTGTGTCTGG GGCCAGGACC TGAACTCCAC GCCTACCTCT 2640
CCATGTTTAC ATGTTCCCAG TATCTTTGCA CAAACCAGGG GTGGGGGAGG GTCTCTGGCT 2700
TCATTTTTCT GCTGTGCAGA ATATTCTATT TTATATTTTT ACATCCAGTT TAGATAATAA 2760
ACTTTATTAT GAAAGTTTTT TTTTTTAAAG AAACAAAGAT TTCTAGA 2807

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 212 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Val Ala Arg Asn Gln Val Glu Ala Asp Asn Ala Ile Ser Pro Ala
1 5 10 15

Ser Glu Pro Arg Arg Arg Pro Glu Pro Ser Ser Ser Ser Ser Ser Ser
20 25 30

Ser Pro Ala Ala Pro Ala Arg Pro Arg Pro Cys Pro Val Val Pro Ala
35 40 45

Pro Ala Pro Gly Asp Thr His Phe Arg Thr Phe Arg Ser His Ser Asp
50 55 60

```
Tyr Arg Arg Ile Thr Arg Thr Ser Ala Leu Leu Asp Ala Cys Gly Phe
65                  70                  75                  80

Tyr Trp Gly Pro Leu Ser Val His Gly Ala His Glu Arg Leu Arg Ser
                85                  90                  95

Glu Pro Val Gly Thr Phe Leu Val Arg Asp Ser Arg Gln Arg Asn Cys
            100                 105                 110

Phe Phe Ala Leu Ser Val Lys Met Ala Ser Gly Pro Thr Ser Ile Arg
        115                 120                 125

Val His Phe Gln Ala Gly Arg Phe His Leu Asp Gly Asn Arg Glu Thr
    130                 135                 140

Phe Asp Cys Leu Phe Glu Leu Leu Glu His Tyr Val Ala Ala Pro Arg
145                 150                 155                 160

Arg Met Leu Gly Ala Pro Leu Arg Gln Arg Arg Val Arg Pro Leu Gln
                165                 170                 175

Glu Leu Cys Arg Gln Arg Ile Val Ala Ala Val Gly Arg Glu Asn Leu
            180                 185                 190

Ala Arg Ile Pro Leu Asn Pro Val Leu Arg Asp Tyr Leu Ser Ser Phe
        195                 200                 205

Pro Phe Gln Ile
    210
```

DATED this 14th day of February 1997

THE WALTER AND ELIZA HALL INSTITUTE
OF MEDICAL RESEARCH
By Its Patent Attorneys
DAVIES COLLISON CAVE

FIGURE 1

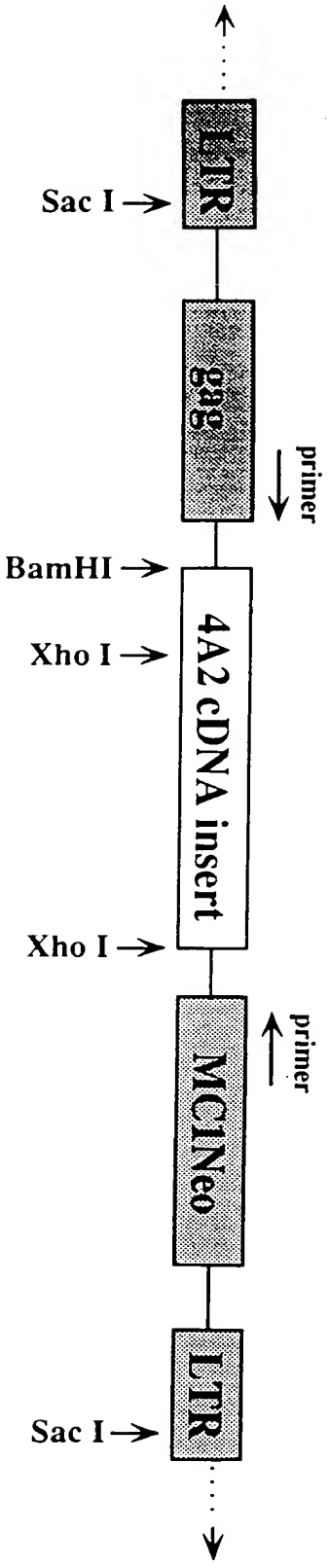

LTR
Sac I
gag
primer
BamHI
Xho I
4A2 cDNA insert
Xho I
primer
MC1Neo
LTR
Sac I

FIGURE 2

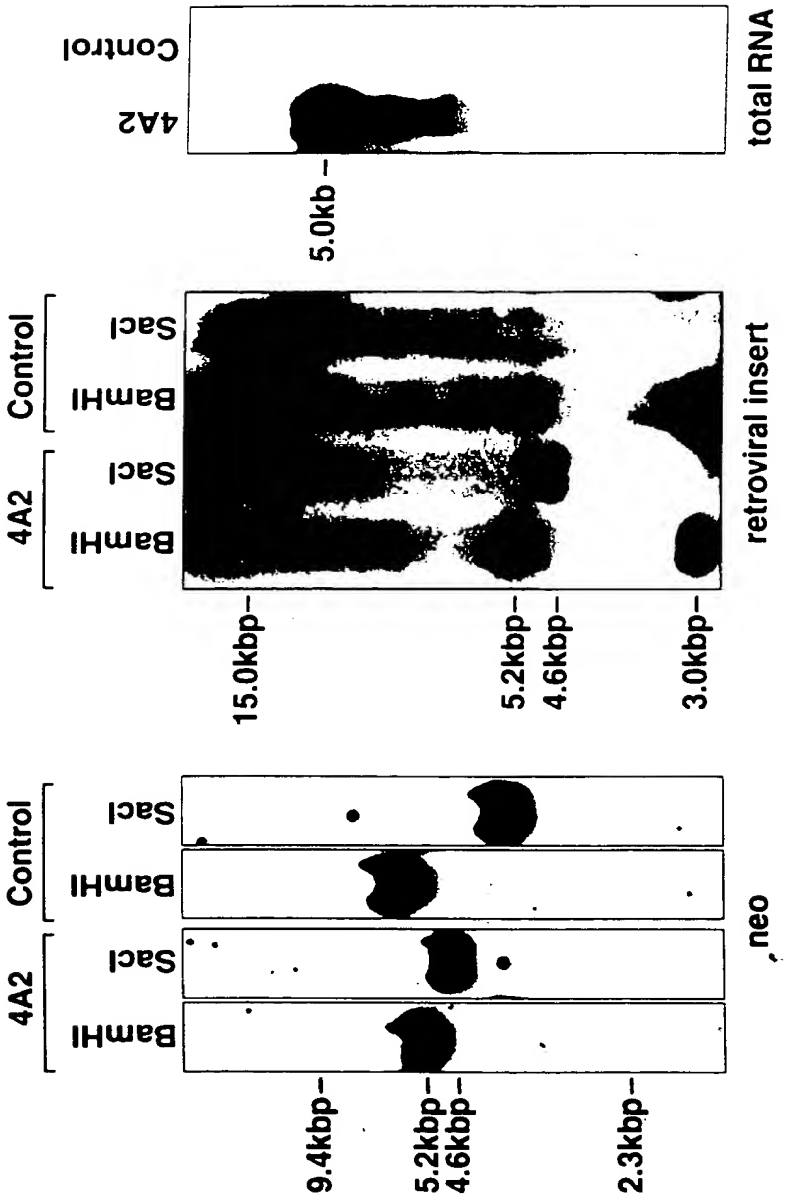
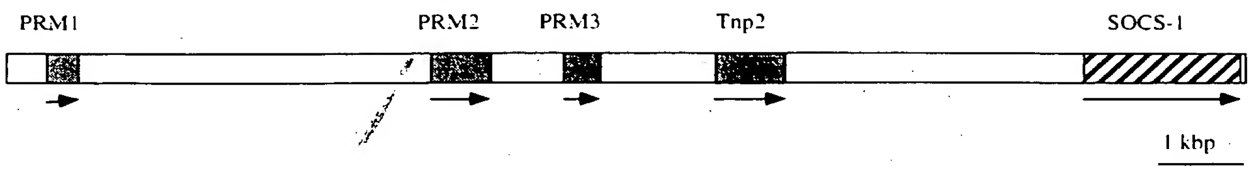

4A2
Control
5.0kb
total RNA
Control
SacI
BamHI
4A2
SacI
BamHI
15.0kbp
5.2kbp
4.6kbp
3.0kbp
retroviral insert
Control
SacI
BamHI
4A2
SacI
BamHI
9.4kbp
5.2kbp
4.6kbp
2.3kbp
neo

FIGURE 3

A



B

```
-133                           cgaggcttaagcttcgggcggattctgcgtgccgctctcg
-120  ctcccttggggttcgttggcccggccttgtgcctacccggacgcccggcttacttgccttcgtct
-60   ccccgattagcgcagccccggacgctatggcccacccccctagctggccccctcgagtagg

1     M  V  A  R  N  Q  V  A  A  D  N  A  I  S  P  A  A  E  P  R
1     ATGGTAGCACGCAACCAGGTGGCAGCCGACAATGCGATCTCCCCGGCAGCAGAGCCCCGA

21    R  R  S  E  P  S  S  S  S  S  S  S  S  P  A  A  P  V  R  P
61    CGGCGGTCAGAGCCCTCCTCGTCCTCGTCTTCGTCCTCGCCAGCGGCCCCCGTGCGTCCC

41    R  P  C  P  A  V  P  A  P  A  P  G  D  T  H  F  R  T  F  R
121   CGGCCCTGCCCGGCGGTCCCAGCCCCAGCCCCTGGCGACACTCACTTCCGCACCTTCCGC

61    S  H  S  D  Y  R  R  I  T  R  T  S  A  L  L  D  A  C  G  F
181   TCCCACTCCGATTACCGGCGCATCACGCGGACCAGCGCGCTCCTGGACGCCTGCGGCTTC

81    Y  W  G  P  L  S  V  H  G  A  H  E  R  L  R  A  E  P  V  G
241   TATTGGGGACCCCTGAGCGTGCACGGGGCGCACGAGCGGCTGCGTGCCGAGCCCGTGGGC

101   T  F  L  V  R  D  S  R  Q  R  N  C  F  F  A  L  S  V  K  M
301   ACCTTCTTGGTGCGCGACAGTCGTCAACGGAACTGCTTCTTCGCGCTCAGCGTGAAGATG

121   A  S  G  P  T  S  I  R  V  H  F  Q  A  G  R  F  H  L  D  G
361   GCTTCGGGCCCCACGAGCATCCGCGTGCACTTCCAGGCCGGCCGCTTCCACTTGGACGGC

141   S  R  E  T  F  D  C  L  F  E  L  L  E  H  Y  V  A  A  P  R
421   AGCCGCGAGACCTTCGACTGCCTTTTCGAGCTGCTGGAGCAcTACGTGGCGGCGCCGCGC

161   R  M  L  G  A  P  L  R  Q  R  R  V  R  P  L  Q  E  L  C  R
481   CGCATGTTGGGGGCCCcGCTGCGCCAGCGCCGCGTGCGGCCGCTGCAGGAGCTGTGTCGC

181   Q  R  I  V  A  A  V  G  R  E  N  L  A  R  I  P  L  N  P  V
541   CAGCGCATCGTGGCCGCCGTGGGTCGCGAGAACCTGGCGCGCATCCcTCTTAACCCGGTA

201   L  R  D  Y  L  S  S  F  P  F  Q  I  *
601   CTCCGTGACTACCTGAGTTCCTTCCCCTTCCAGATCtgaccggctgccgctgtgccgcag

661   cattaagtgggggcgccttattatttcttattattaattattattatttttctggaacca
721   cgtgggagccctccccgcctgggtcggagggagtggttgtggagggtgagatgcctccca
781   cttctggctggagacctcatcccacctctcaggggtgggggtgctcccctcctggtgctc
841   cctccgggtccccccctggttgtagcagcttgtgtctggggccaggacctgaattccactc
901   ctacctctccatgtttacatattcccagtatctttgcacaaaccaggggtcggggagggt
961   ctctggcttcatttttctgctgtgcagaatatcctatttatatttttacagccagttta
1021  ggtaataaactttattatgaagttttttttaaaagaaaaaaaaaaaaaaaaa
```

(underlined in original: aataaa at 1025–1030)

FIGURE 4

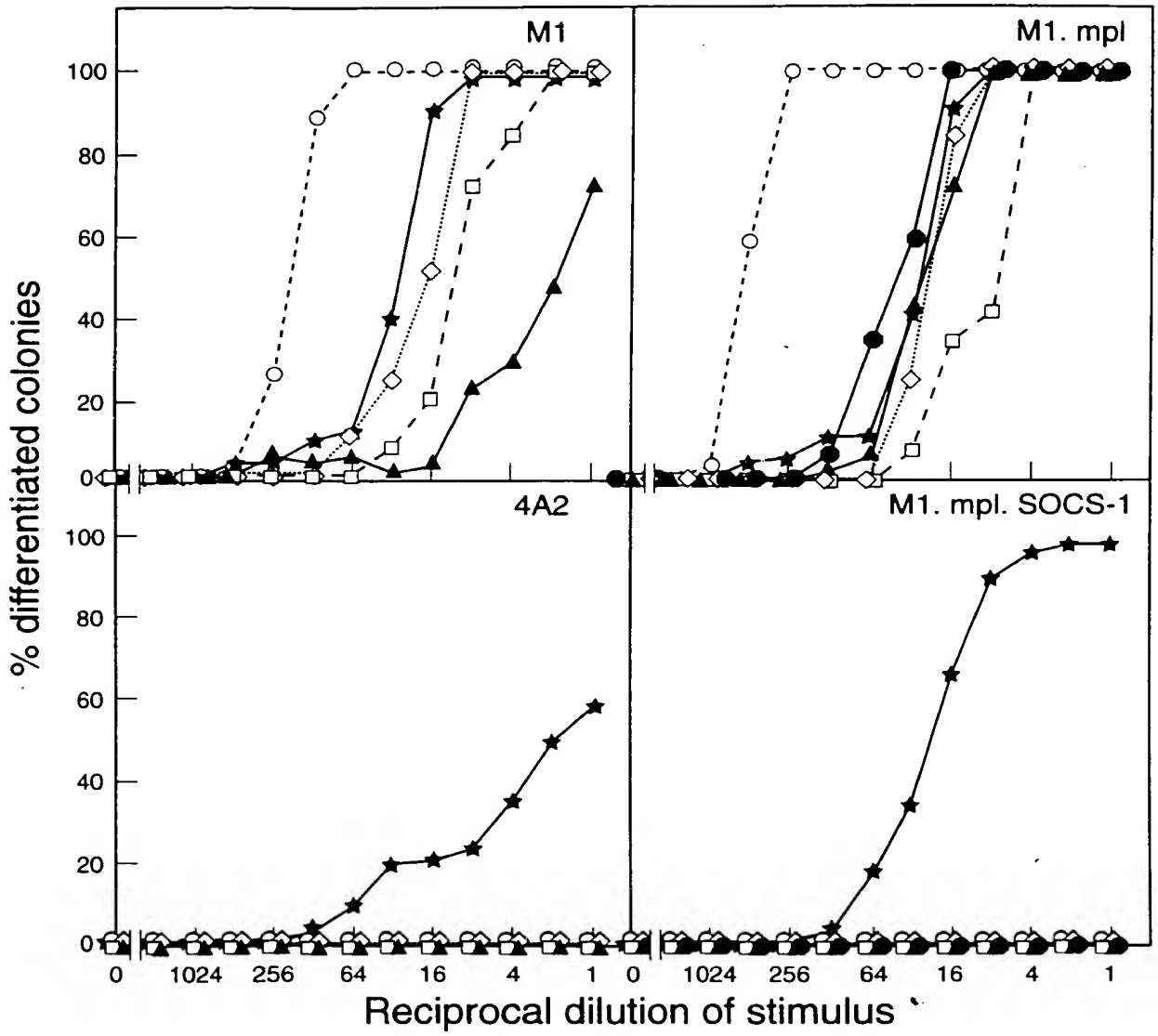

M1
M1. mpl
4A2
M1. mpl. SOCS-1
% differentiated colonies
100
80
60
40
20
0
0 1024 256 64 16 4 1
Reciprocal dilution of stimulus

FIGURE 5

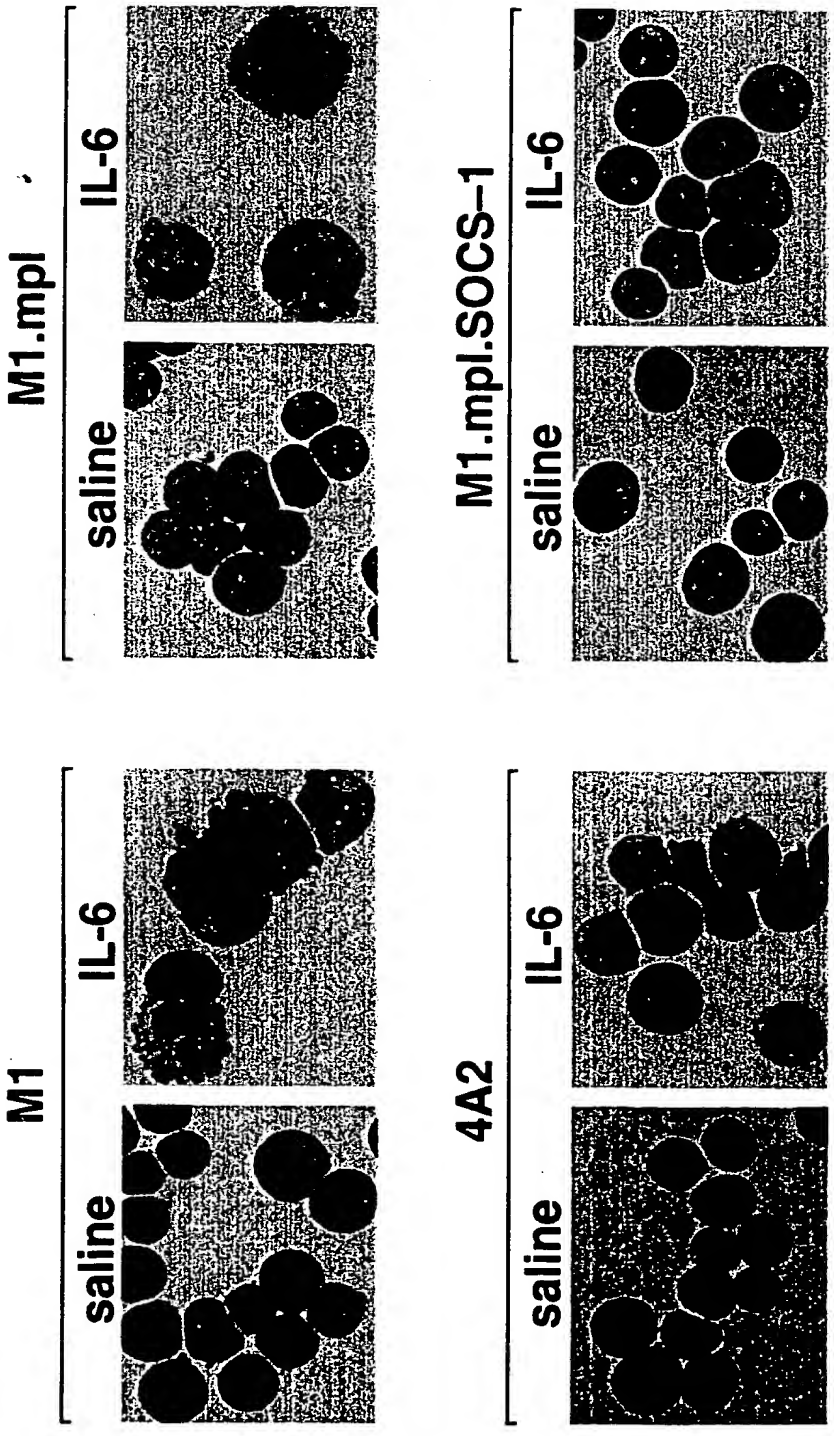

M1.mpl
IL-6
saline
M1.mpl.SOCS-1
IL-6
saline
M1
IL-6
saline
4A2
IL-6
saline

FIGURE 6

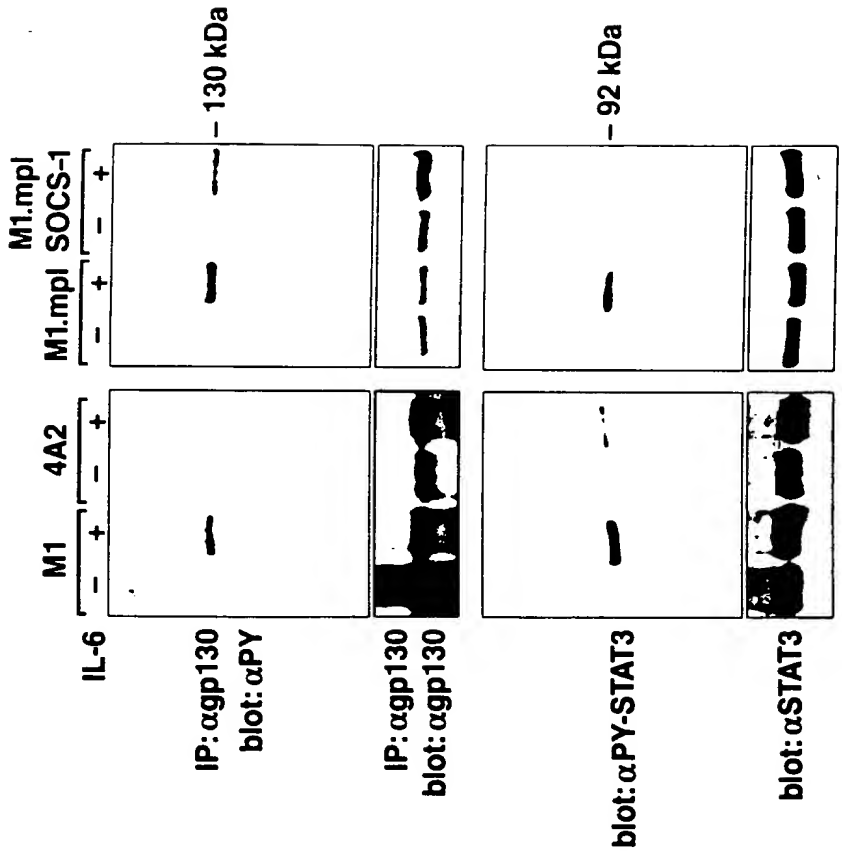

IL-6
M1
4A2
M1.mpl
M1.mpl
SOCS-1
IP: αgp130
blot: αPY
IP: αgp130
blot: αgp130
blot: αPY-STAT3
blot: αSTAT3
130 kDa
92 kDa

FIGURE 7A

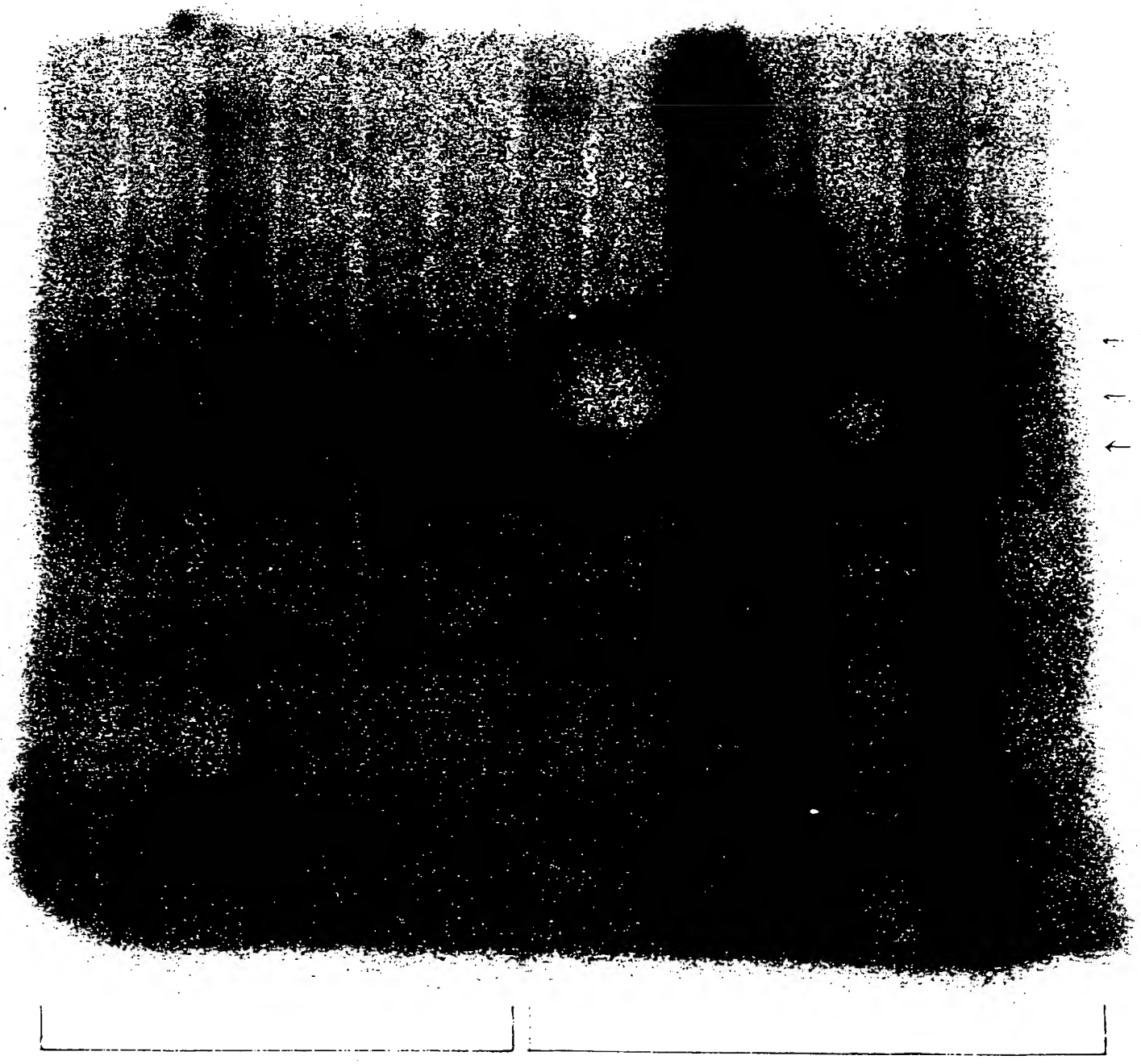


FIGURE 7B

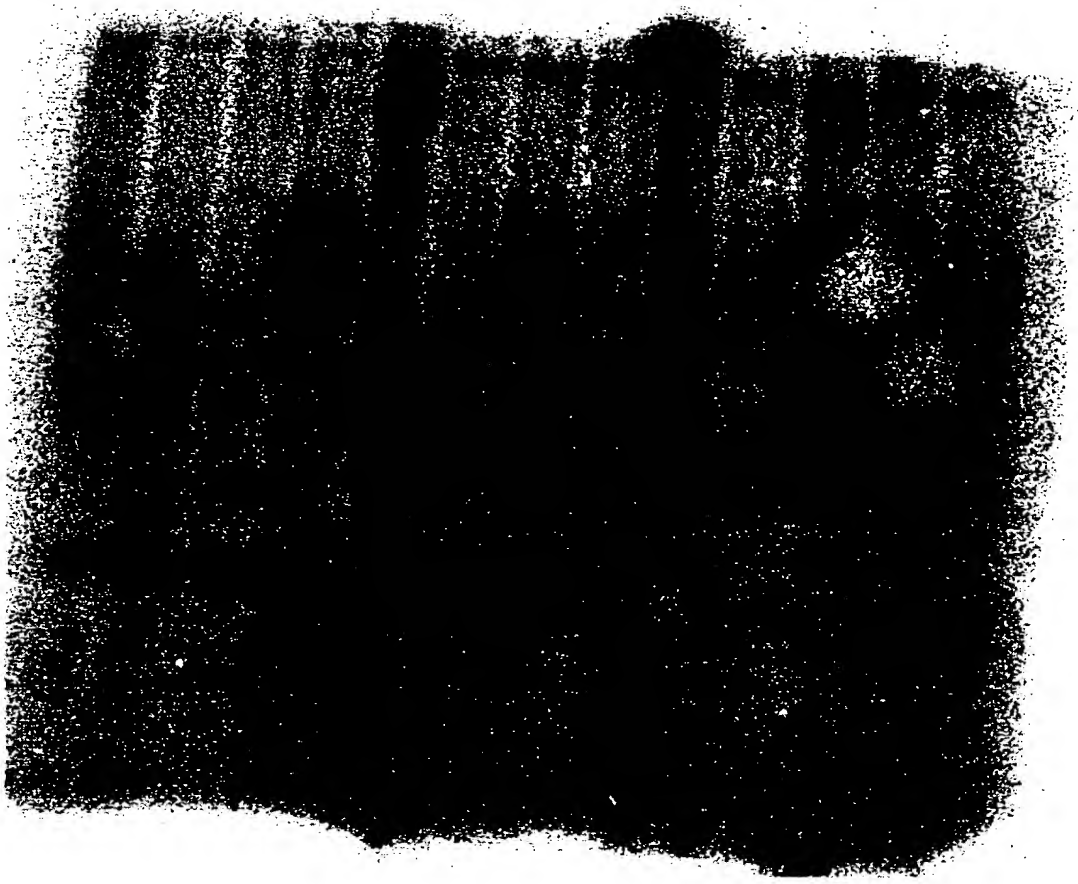
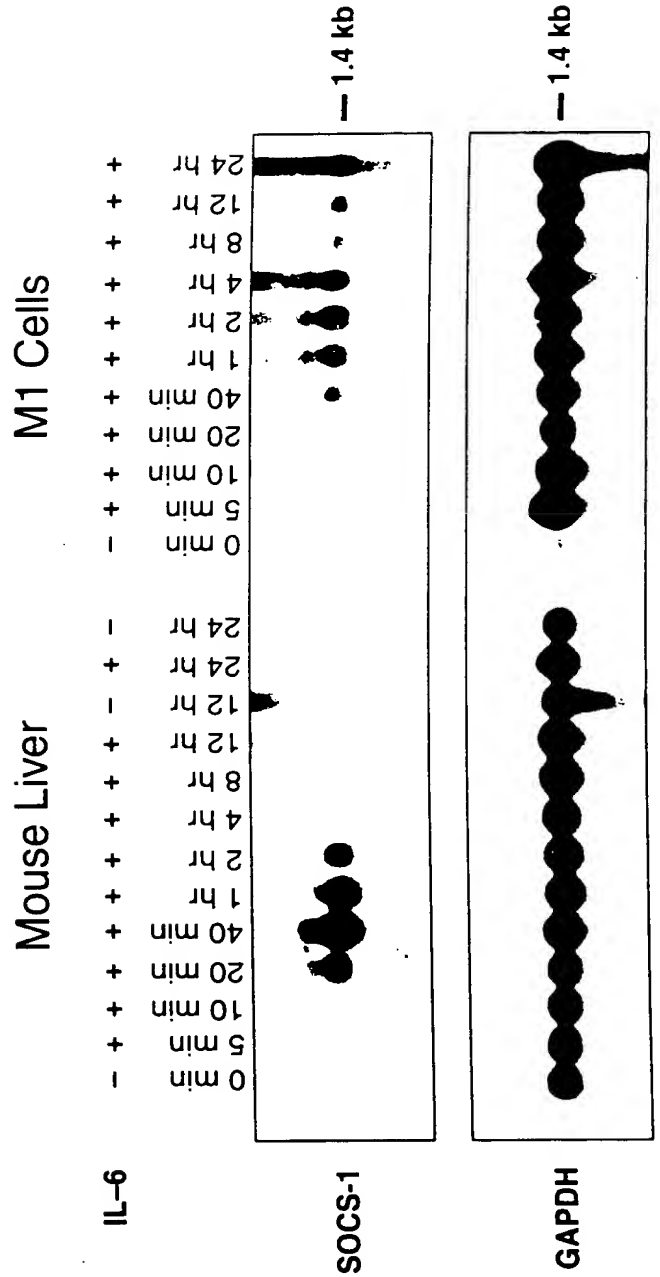


FIGURE 8


Mouse Liver
M1 Cells
IL-6
0 min -
5 min +
10 min +
20 min +
40 min +
1 hr +
2 hr +
4 hr +
8 hr +
12 hr +
12 hr -
24 hr +
24 hr -
0 min -
5 min +
10 min +
20 min +
40 min +
1 hr +
2 hr +
4 hr +
8 hr +
12 hr +
24 hr +
SOCS-1
GAPDH
1.4 kb
1.4 kb

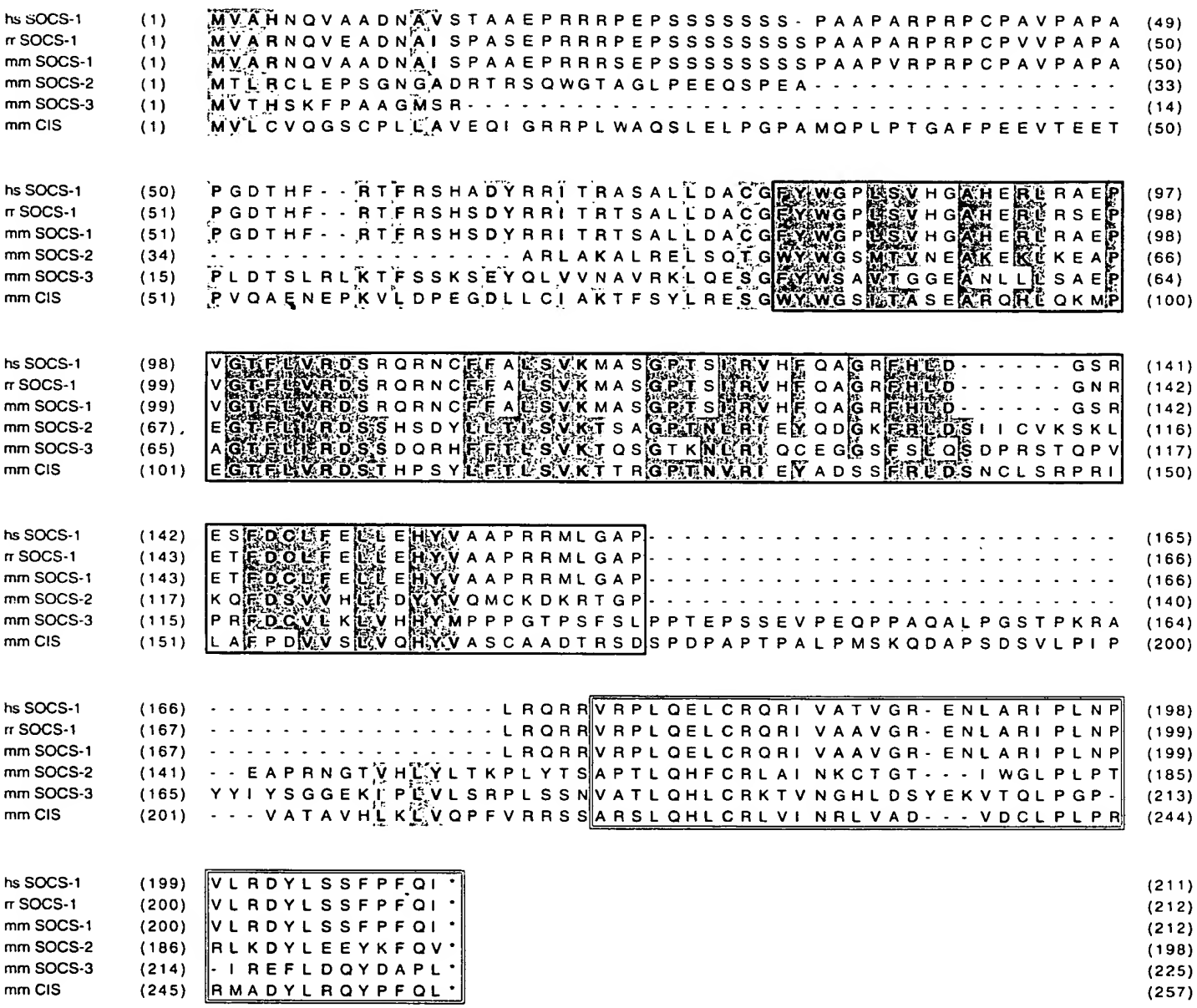

hs SOCS-1 (1) MVAHNQVAADNAVSTAAEPRRRPEPSSSSSSS-PAAPARPRPCPAVPAPA (49)
rr SOCS-1 (1) MVARNQVEADNAISPASEPRRRPEPSSSSSSSSSPAAPARPRPCPVVPAPA (50)
mm SOCS-1 (1) MVARNQVAADNAISPAAEPRRRSEPSSSSSSSSSPAAPVRPRPCPAVPAPA (50)
mm SOCS-2 (1) MTLRCLEPSGNGADRTRSQWGTAGLPEEQSPEA---------- (33)
mm SOCS-3 (1) MVTHSKFPAAGMSR---------- (14)
mm CIS (1) MVLCVQGSCPLLAVEQIGRRPLWAQSLELPGPAMQPLPTGAFPEEVTEET (50)
hs SOCS-1 (50) PGDTHF--RTFRSHADYRRITRASALLDACGFYWGPLSVHGAHERLRAEP (97)
rr SOCS-1 (51) PGDTHF--RTFRSHSDYRRITRTSALLDACGFYWGPLSVHGAHERLRSEP (98)
mm SOCS-1 (51) PGDTHF--RTFRSHSDYRRITRTSALLDACGFYWGPLSVHGAHERLRAEP (98)
mm SOCS-2 (34) ----------ARLAKALRELSQTGWYWGSMTVNEAKEKLKEAP (66)
mm SOCS-3 (15) PLDTSLRLKTFSSKSEYQLVVNAVRKLQESGFYWSAVTGGEANLLLSAEP (64)
mm CIS (51) PVQAENEPKVLDPEGDLLCIAKTFSYLRESGWYWGSLTASEARQHLQKMP (100)
hs SOCS-1 (98) VGTFLVRDSRQRNCFFALSVKMASGPTSIRVHFQAGRFHLD------GSR (141)
rr SOCS-1 (99) VGTFLVRDSRQRNCFFALSVKMASGPTSIRVHFQAGRFHLD------GNR (142)
mm SOCS-1 (99) VGTFLVRDSRQRNCFFALSVKMASGPTSIRVHFQAGRFHLD------GSR (142)
mm SOCS-2 (67) EGTFLIRDSSHSDYLLTISVKTSAGPTNLRIEYQDGKFRLDSIICVKSKL (116)
mm SOCS-3 (65) AGTFLIRDSSDQRHFFTLSVKTQSGTKNLRIQCEGGSFSLQSDPRSTQPV (117)
mm CIS (101) EGTFLVRDSTHPSYLFTLSVKTTRGPTNVRIEYADSSFRLDSNCLSRPRI (150)
hs SOCS-1 (142) ESFDCLFELLEHYVAAPRRMLGAP---------- (165)
rr SOCS-1 (143) ETFDCLFELLEHYVAAPRRMLGAP---------- (166)
mm SOCS-1 (143) ETFDCLFELLEHYVAAPRRMLGAP---------- (166)
mm SOCS-2 (117) KQFDSVVHLIDYYVQMCKDKRTGP---------- (140)
mm SOCS-3 (115) PRFDCVLKLVHHYMPPPGTPSFSLPPTEPSSEVPEQPPAQALPGSTPKRA (164)
mm CIS (151) LAFPDVVSLVQHYVASCAADTRSDSPDPAPTPALPMSKQDAPSDSVLPIP (200)
hs SOCS-1 (166) ----------LRQRRVRPLQELCRQRIVATVGR-ENLARIPLNP (198)
rr SOCS-1 (167) ----------LRQRRVRPLQELCRQRIVAAVGR-ENLARIPLNP (199)
mm SOCS-1 (167) ----------LRQRRVRPLQELCRQRIVAAVGR-ENLARIPLNP (199)
mm SOCS-2 (141) --EAPRNGTVHLYLTKPLYTSAPTLQHFCRLAINKCTGT---IWGLPLPT (185)
mm SOCS-3 (165) YYIYSGGEKIPLVLSRPLSSNVATLQHLCRKTVNGHLDSYEKVTQLPGP- (213)
mm CIS (201) ---VATAVHLKLVQPFVRRSSARSLQHLCRLVINRLVAD---VDCLPLPR (244)
hs SOCS-1 (199) VLRDYLSSFPFQI* (211)
rr SOCS-1 (200) VLRDYLSSFPFQI* (212)
mm SOCS-1 (200) VLRDYLSSFPFQI* (212)
mm SOCS-2 (186) RLKDYLEEYKFQV* (198)
mm SOCS-3 (214) -IREFLDQYDAPL* (225)
mm CIS (245) RMADYLRQYPFQL* (257)

FIGURE 10

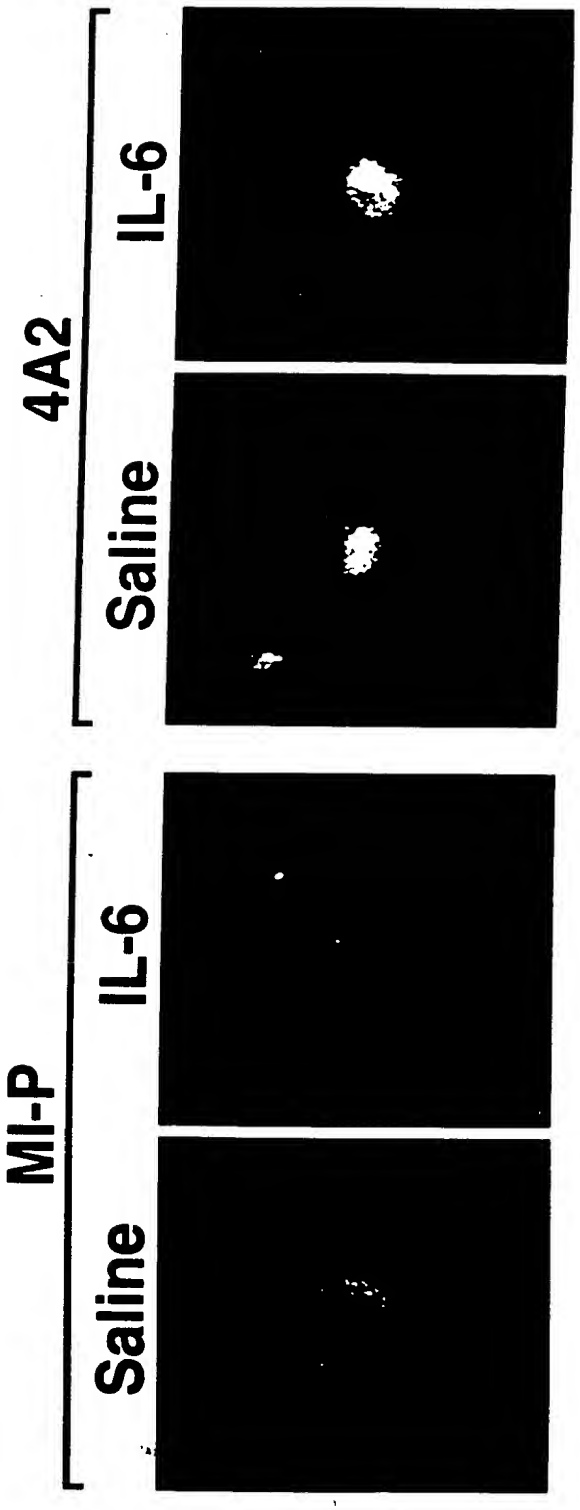


FIGURE 11

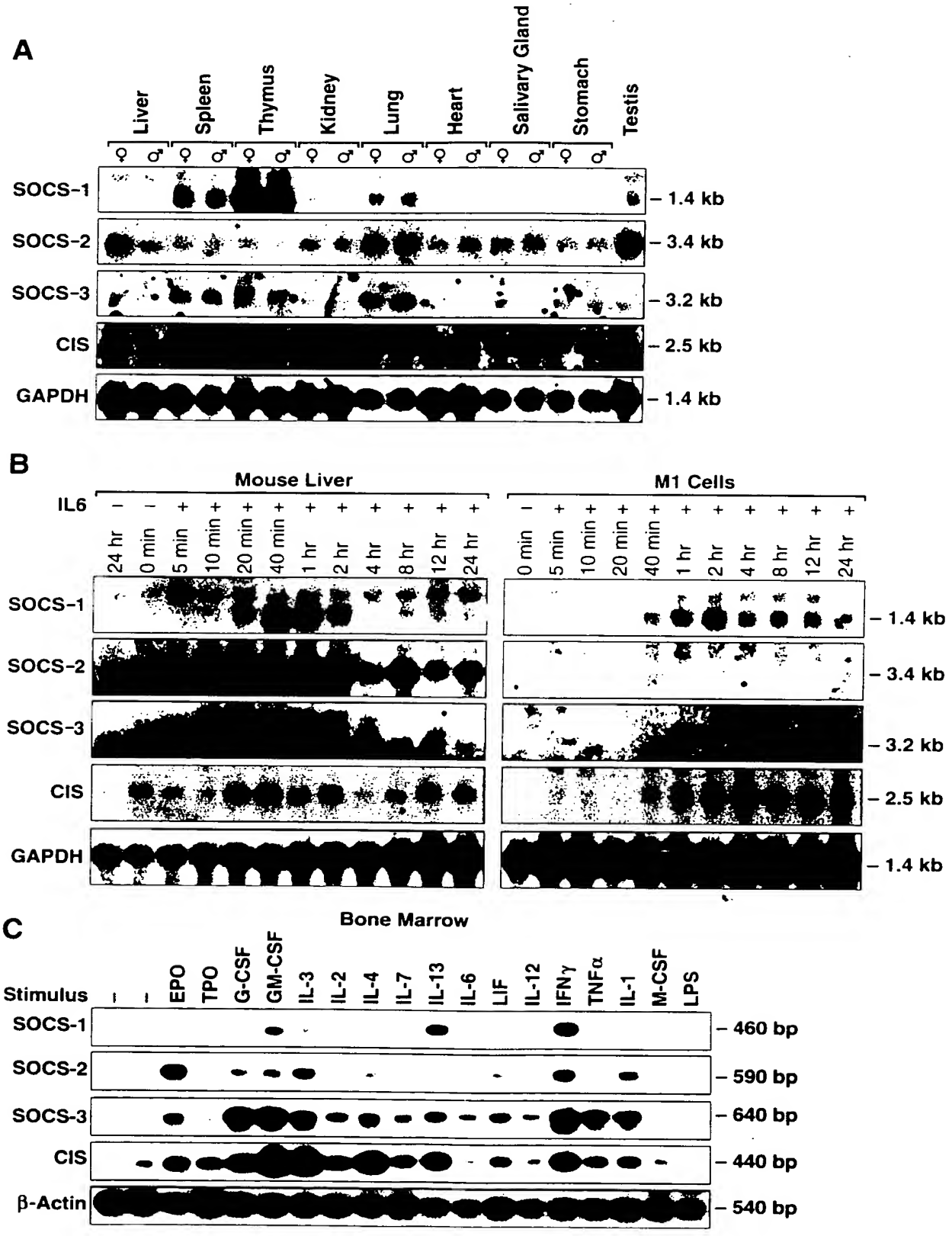
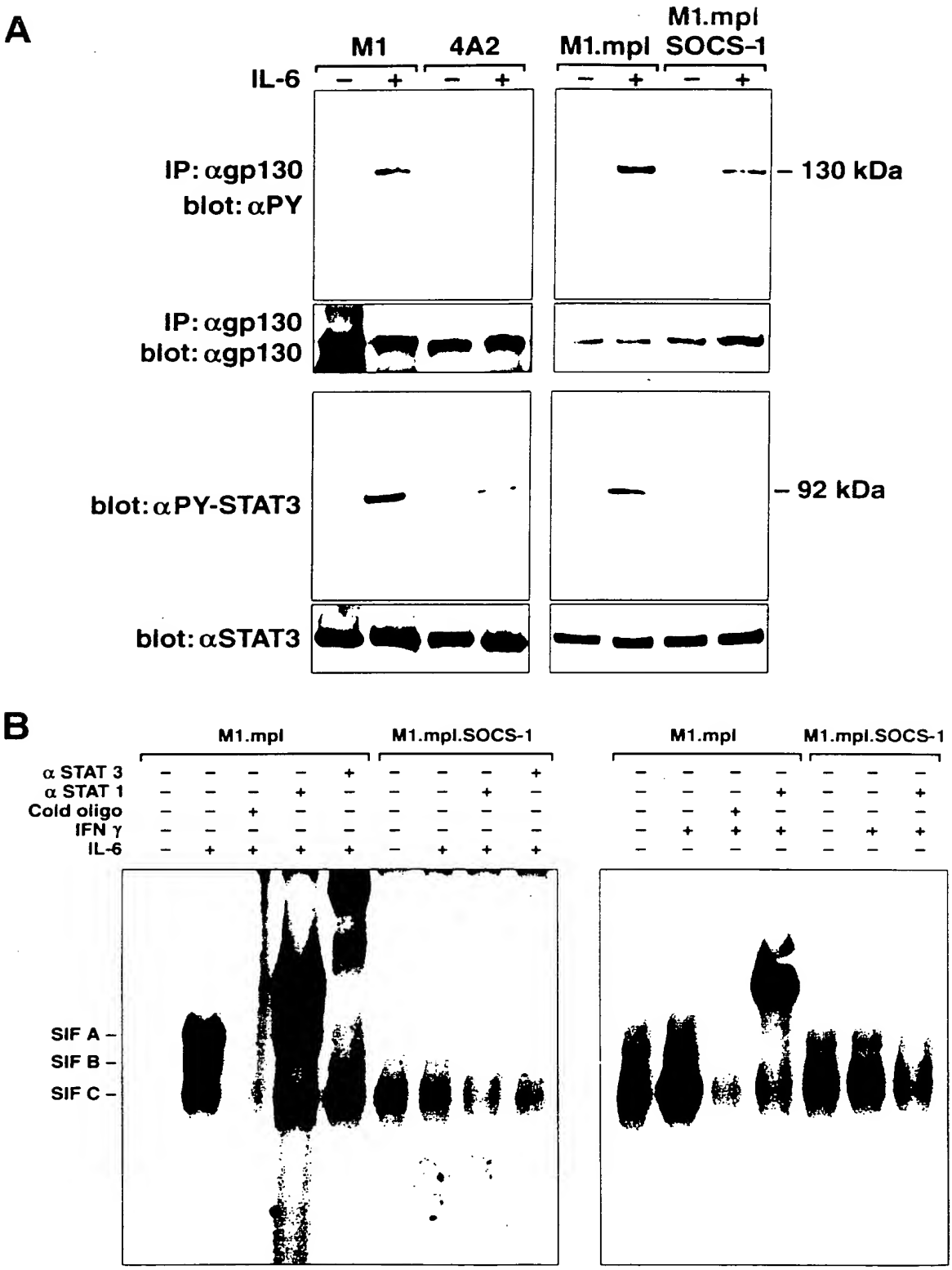

A
Liver
Spleen
Thymus
Kidney
Lung
Heart
Salivary Gland
Stomach
Testis
♀ ♂
SOCS-1
SOCS-2
SOCS-3
CIS
GAPDH
– 1.4 kb
– 3.4 kb
– 3.2 kb
– 2.5 kb
– 1.4 kb
B
Mouse Liver
M1 Cells
IL6
– – + + + + + + + + + +
24 hr 0 min 5 min 10 min 20 min 40 min 1 hr 2 hr 4 hr 8 hr 12 hr 24 hr
– + + + + + + + + + +
0 min 5 min 10 min 20 min 40 min 1 hr 2 hr 4 hr 8 hr 12 hr 24 hr
SOCS-1
SOCS-2
SOCS-3
CIS
GAPDH
– 1.4 kb
– 3.4 kb
– 3.2 kb
– 2.5 kb
– 1.4 kb
C
Bone Marrow
Stimulus
– – EPO TPO G-CSF GM-CSF IL-3 IL-2 IL-4 IL-7 IL-13 IL-6 LIF IL-12 IFNγ TNFα IL-1 M-CSF LPS
SOCS-1
SOCS-2
SOCS-3
CIS
β-Actin
– 460 bp
– 590 bp
– 640 bp
– 440 bp
– 540 bp

FIGURE 12


A
M1
4A2
M1.mpl
M1.mpl SOCS-1
IL-6
– + – + – + – +
IP: αgp130
blot: αPY
– 130 kDa
IP: αgp130
blot: αgp130
blot: αPY-STAT3
– 92 kDa
blot: αSTAT3
B
M1.mpl
M1.mpl.SOCS-1
M1.mpl
M1.mpl.SOCS-1
α STAT 3
α STAT 1
Cold oligo
IFN γ
IL-6
SIF A –
SIF B –
SIF C –